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TWO MUTATORS OF *SACCHAROMYCES CEREVISIAE*: *mut7* AND *mut8*

by

©

ROBIN WYNNE ORD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Two mutators of *Saccharomyces cerevisiae: mut7* and *mut8*

submitted by Robin Wynne Ord

in partial fulfilment of the requirements for the degree of Master of Science

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ABSTRACT

Two mutator mutants of yeast, *mut7-1* and *mut8-1* were found to segregate from a mutator strain which had been isolated on the basis of enhanced spontaneous mutation following EMS mutagenesis (von Borstel and Quah, unpublished data). The double mutator mutant was shown to confer a large enhancement of spontaneous mutation at many loci. This enhancement was much greater than the sum of the mutator activities of *mut7-1* or of *mut8-1* alone; these latter confer low mutation rates which are often comparable with non-mutator strains of yeast.

The *mut7-1* allele was shown to confer a temperature-sensitive-lethal phenotype. This phenotype co-reverted and co-segregated with the following phenotypes: sensitivity to MMS, enhanced spontaneous intragenic recombination, and the original mutator activity.

The *mut8-1*-mediated mutator activity was found to be associated with increased numbers of (*his1-7*) revertants which arose in G1, stationary phase cultures, following exponential cell growth. The temperature-sensitivity of *mut7* mutants causes cells to arrest in the G1 stage of the yeast cell cycle at the restrictive temperature. The large enhancement of spontaneous mutation seen in *mut7mut8* strains at the permissive temperature might be caused by the interaction, in G1, of the phenotypes conferred by both mutators.

Crosses of *mut7-1* or of *mut8-1* to the mutators *mut1-1*, *mut2-1*, *mut3-1*, *mut4-1*, *mut5-1*, *mut6-1*, *mut9-1*, and *rad52-1* has established that *mut7* and *mut8* are not allelic with these mutator loci. Four different phenotypes were noted in double mutator mutant spore clones segregating from these crosses. Mutator activity in many double mutator mutants was additive in terms of the mutator activities shown

by the separate mutators (as indicated by revertant frequencies for the auxotrophies caused by *lys1-1* or *his1-7*), which implies that those mutators did not interact. In other double mutator mutant strains, the mutator activity was synergistically enhanced over the separate activities. In some combinations, the mutator activity of one mutator could account for the numbers of revertants observed in a double mutator mutant (an epistatic interaction). The fourth phenotype noted in segregating double mutator mutant spores was spore lethality.

The interactions between mutators are discussed in terms of the channelling theory of spontaneous mutagenesis, and in terms of spontaneous lesions introduced into DNA during its repliction or repair.

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INTRODUCTION

The generation of spontaneous mutations in yeast has been extensively examined by von Borstel *et al.* (1973), Hastings *et al.* (1976) and Quah (1979). These studies have correlated alterations in spontaneous mutation rates with changes in the cell's ability to repair spontaneously occurring lesions in DNA with high fidelity, in two ways. Mutants defective in the repair of radiation damage were found to have alterations in spontaneous mutagenesis (Hastings *et al.*, 1976). Mutator (*mut*) mutants (von Borstel *et al.*, 1973) or antimutator (*ant*) mutants (Quah, 1979) isolated on the basis of enhanced or reduced spontaneous mutation, respectively, frequently had alterations in DNA repair parameters, such as radiation sensitivity (Hastings *et al.*, 1976, Morrison, 1978). In at least two instances, the mutants isolated for altered spontaneous mutator activity have been found to be allelic with mutants isolated on the basis of defects in the repair of radiation damage. [For example, the *rev3-1* allele (isolated on the basis of reduced levels of UV-induced mutation; Lemontt, 1971) was found to be allelic with *ant2-1*, isolated by Quah and Lynch (unpublished). Similarly, *mut5-1* was found to be allelic with the radiation sensitive (*rad*) mutant, *rad51-1* (Morrison, 1978).]

In spite of these findings, the sources of spontaneous *lesions* in yeast DNA (as the substrates for spontaneous mutagenesis) remain unknown, as do the mechanisms by which these lesions are processed into mutations in this organism. However, there are but four mechanisms known for causing a spontaneous mutation: replication of base mispairs in DNA, repair of base mispairs, and insertions and deletions of bases. The

latter two have been shown to be the major sources of spontaneous mutation in the *lac* I gene of *E. coli* (Farabaugh *et al.*, 1978). However, since deletions and insertions of more than one base normally occur infrequently in yeast (but see Klar *et al.*, 1979), these will not be considered as major sources of spontaneous mutations in this thesis.

A. Replication of Base Mispairs in DNA

In the absence of repair processes, a replicable mispair in the double stranded DNA, whatever its source, may be replicated to create two new duplex DNA molecules, each differing by one base pair. The categories of base mispairs are numerous. C-A, T-G, Py-Py (opposing pyrimidines) and Pu-Pu (opposing purines) are mispairs (see Topal and Fresco, 1976, for mechanisms by which these may arise) created by unaltered bases misincorporated into DNA. Tautomeric shifts were proposed by Watson and Crick (1956) to cause Py-Pu mispairs. Further types of mispairs can be caused by pyrimidine dimers (reviewed by Witkin, 1976), insertion of base analogues with altered keto-enol ratios (Kirchner, 1960), alkylation of bases in DNA (reviewed by Cerutti, 1975), and deletions or additions of one base in one strand of the duplex (frameshifts; Magni 1963; Magni and von Borstel, 1962; Streisinger *et al.*, 1966) such as might be induced by intercalating agents (Ames and Whitfield, 1966) or by A-T or G-C runs (Streisinger *et al.*, 1966). It is generally believed that semi-conservative replication will not occur opposite pyrimidine dimers. However, (repair) polymerization of bases opposite such non-replicating lesions could occur (see Witkin).

Spontaneous mispairs may arise as a function of DNA synthesis, from tautomeric shifts just prior to replication (Watson and Crick, 1956), from polymerase errors (Speyer, 1965), from poor editing of replicated base mispairs (Goulian, Lucas and Kornberg, 1968; Hershfield and Nossal, 1973), from alterations in the cell-mediated modification of DNA (Marinus and Morris, 1974; Glickman, 1978) or from modified DNA *per se* Coulondre *et al.*, 1978). Cell-mediated mispairing of DNA might also arise during generalized recombination (meiotic, conjugative or mutagen induced), by the bringing together of homologous DNA strands from different chromosomes to create heteroduplex DNA (Holliday, 1962). These strands might differ in at least one base pair due to Py-Pu, Pu-Pu, Py-Py mispairs, or due to frameshift mutations (Magni, 1963).

B. The Mutagenic Repair of Spontaneous Lesions in DNA

Since many of the induced mispairs noted above are excised by cellular repair mechanisms (for an excellent review, see "Molecular Mechanisms for the Repair of DNA", 1975, ed. Hanawalt and Setlow, Plenum Press) it would seem natural for spontaneously occurring lesions in DNA to be removed in the same way. Hastings *et al.* (1976) proposed that such spontaneous lesions were not free to segregate into daughter cells as mutations without having been first subjected to cellular repair processes. The proposal was based on the observations mentioned above, and upon the pathway hypothesis for UV-induced mutation first proposed by Witkin (1969). This hypothesis has been used to categorize repair of UV-damage in yeast by Cox and Game (1974), who proposed that primary lesions induced in yeast nuclear DNA by UV-irradiation may be

repaired in one of three ways: by excision repair, recombination repair or mutagenic repair. The first two pathways have been more clearly resolved than the third, which has been defined mainly by mutants which reduce UV-induced mutagenesis, or by the increased UV-induced mutation frequencies of excision-repair defective mutants (for reviews see Morrison, 1978; Lawrence and Christensen, 1976; Haynes, 1975).

A large number of *rad* mutants have been isolated by different experimenters (see Game, 1975). These have all been considered to be blocked in one of the three modes of repair described. Mutants of excision repair tend to be sensitive to ultraviolet (UV) irradiation and not sensitive to γ or x-irradiation compared to RAD^+ strains. Mutants of recombination repair are usually more sensitive to γ-irradiation than to U.V. irradiation.

When one of three pathways is blocked in a *rad* mutant, UV-induced lesions may be channelled into one or both of the other two. The mutations derived from the primary lesions (base mispairs caused by pyrimidine dimers, in the context described above) would be expected to increase or to decrease, depending on which pathway had been blocked.

Hastings *et al.* (1976) contended that the same might be true of spontaneous mutation. Hence, if one non-mutagenic repair pathway for spontaneously occurring mispairs in DNA were blocked by mutation in a yeast strain, that mispair would be channelled more frequently into the mutagenic repair pathway, and so the strain would be a mutator strain.

One prediction of this hypothesis, based on results from UV-induced mutagenesis (Cox and Game, 1974), was that two repair mutants, *a* and *b*, which affect the same non-mutagenic repair pathway for a spontaneous

mispair, would be epistatic for enhanced mutation rate in the double mutant *a,b*. It was also implied that a second type of epistasis was likely to appear. If a mutation in a repair gene resulted in the blocking of the mutagenic repair pathway, that mutation would be epistatic to another repair mutation that caused enhanced spontaneous mutagenesis by the channelling of mispairs into mutagenic repair. The original spontaneous lesion might use another non-mutagenic pathway, If all pathways were blocked, the mispair might segregate into a daughter cell as a mutation in any case, or it might kill the cell.

Another prediction, also based on the pathway hypothesis, would be that if the two non-mutagenic repair pathways competing for one spontaneous lesion were blocked, the number of lesions using the third, mutagenic pathway would be synergistically enhanced. The spontaneous mutation rate should therefore increase drastically.

This implication of DNA repair in spontaneous mutagenesis does not refute the replication-mutagenesis hypotheses. It remains to be shown that spontaneous mutagenic repair is not a cell-mediated ability to replicate a mispair, or to ignore a mispair created by replication.

C. The Isolation of Mutators in Yeast

It was in the context of examining all possible sources of spontaneous mutations that the mutator strains of yeast or of *E. coli* were originally isolated (von Borstel *et al.*, 1971; for a review of *E. coli* mutators, see Cox, 1976). In the case of the yeast mutators the aforementioned correlation of mutator activity with defects in the repair of induced lesions in DNA was soon noted. Since the proposal of Hastings -

et al. (1976), that spontaneous mutation may be mediated by mutagenic repair mechanisms, Quah (1979) has identified at least one such pathway in yeast. The *rev3-1* allele is a spontaneous antimutator (Quah, unpublished data). This allele is epistatic to *rad3-1* and to *rad18-1* in double mutant combinations. Normally, *rad3-1* and *rad18-1* both confer a mutator phenotype. Each is thought to block one of the aforementioned repair pathways. Strains bearing *rev3rad3*, or *rev3rad18* have low spontaneous mutation rates resembling those of *rev3* strains.

From these results, mutator loci of yeast may now be considered either as sources of spontaneous lesions to be handled by cellular mutagenic repair systems (whether or not their respective MUT^+ alleles are involved in repair mechanisms), or as possibly defective repair loci.

By utilizing the 100-compartment fluctuation test for *lys1-1* locus reversion and *lys1-1* suppressor mutations (*lys1-1* is an ochre allele), and for *his1-7* reversion, von Borstel *et al.* (1971), Gottlieb and von Borstel (1976) and Quah and von Borstel (unpublished results) have defined ten mutator loci (*mut1* to *mut10*), that confer mutator activity at these loci. The integration of these mutator loci into the general theory of yeast repair has already been initiated by Hastings *et al.* (1976), Morrison (1978), Nasim and Brychcy (1979) and Quah (1979). This study reports the phenotypes of two mutator alleles, *mut7-1* and *mut8-1*, and novel interactions of these alleles with other mutator mutants in double mutator mutant strains.

D. *mut7* and *mut8*

Of the original mutator alleles isolated by von Borstel *et al.* (1971),

mut7-1 and *mut8-1* seemed to have exceptional properties. The two alleles were isolated in one strain following treatment with ethylmethanesulfonate. This strain had one of the highest mutation rates observed in any of the mutator strains (Quah and von Borstel, unpublished). In addition, and unlike many of the other mutator loci, the strain showed greatly enhanced numbers of *lys1-1* locus revertants, as opposed to suppressors (of this ochre allele).

It was not clear how this strongest of mutator strains could be incorporated into any of the aforementioned repair or replication schemes. The fact that neither *mut7* nor *mut8* alone conferred the large enhancement of spontaneous mutation seen in *mut7 mut8* strains seemed to conform to one aspect of the channelling hypothesis: the blocking of two pathways which normally compete for the same spontaneous lesion will synergistically enhance the number of lesions using a third (mutagenic) pathway.

An attempt to obtain an understanding of these mutators in terms of the existing theoretical framework for spontaneous mutagenesis evolved into three areas of study.

The first step was the independent examination of the two mutators contributing to the *mut7 mut8*-mediated mutator activity, for pleiotropic effects of these loci on strains bearing them.

The second step was to examine the separate loci in haploid strains also bearing other mutator alleles (e.g., *mut8 mut1*). This was to determine that *mut7* and *mut8* were not allelic with other known mutators, and to test whether such double mutator mutants would confer epistatic, additive or synergistic enhancements in spontaneous mutation rate when compared with either single mutator allele. Synergism will be defined as any

greater-than-additive enhancement of mutation rate. Additivity means that the sum of the rates conferred by the separate mutators are equal to the rate seen in the double mutant, while epistasis means that the rate of the double mutant equals that of one of the single mutants. Additivity of mutation rates (which implies that two processes contribute independently to the spontaneous mutation rate observed) was considered to be the null hypothesis of no interaction between different mutator loci.

The third line of investigation was derived from the second. We wanted to ask whether additive, epistatic or synergistic interactions of mutator loci would be the same regarding alterations in spontaneous mutation at different test loci. Revertants of the auxotrophies *his1-7* and *lys1-1* were compared since these have been well characterized in previous studies (von Borstel *et al.*, 1971; Flury *et al.*, 1976; and Gottlieb and von Borstel, 1976).

MATERIALS AND METHODS

A. Strains

All mutator mutants studied here were induced in strain X1687-12B (obtained from R.K. Mortimer) by von Borstel *et al.* (1971), with the exception of radiation sensitive (*rad*) strains bearing *rad51-1* isolated by Nakai and Matsumato (1967), or *rad52-1*, isolated by Resnick, 1969). All mutator stocks have been crossed at least twice to non-mutator strains prior to this study.

Table 1 shows the genotypes of strains used to construct stocks. a and α identify mating type alleles. The abbreviations *his*, *lys*, *hom*, *ade*, *trp*, *arg*, *leu*, and *ura* designate recessive alleles whose presence (in haploids) results in auxotrophy for histidine, lysine, homoserine, adenine, tryptophan, arginine, leucine and uracil, respectively. Cryptopleurine resistance is designated by *cry*. Mutator loci are identified by the abbreviation *mut*. The particular locus and allele number are identified by the numbers following the three-letter designation. A "+" in italics following the latter indicates that the allele or locus does not confer a mutant phenotype for the trait concerned.

Genetic manipulations of *mut7-1* strains and of revertant alleles (e.g. *mut7-1-11*) are summarized in Table 2 (and in the Appendix, Figure A1).

Table 3 shows strains employed to eliminate genetically *ts* markers (other than *mut7*) from each mutator background genotype. Table 4 indicates the mutator strains used to cross to the *mut7* strain

RO428-6B, for allelism testing and subsequent tetrad analysis of each double mutator heterozygote synthesized. Strain RO428-6B^{r} carries a revertant of *mut7-1*, allele *mut7-1-11*. Table 5 indicates the mutator strains used to cross to the *mut8-1* strain R088-1C for the same purposes.

The construction of stocks bearing only *his1-7* and combinations of *mut7* and/or *mut8* for studies of forward mutation to auxotrophy is illustrated by Table 6.

Table 7 gives the genotypes of tester strains used to determine genotypes for a or α alleles, for *his*$^{-}$ auxotrophs, and for the mutators. The LZ13 and RO122 strains were employed as tester strains for *his1* allele determination.

The double mutant mutator haploid strains shown in Table 8 are those which have been confirmed by outcrossing to the *mut* testers shown in Table 7. The procedure employed is illustrated by the following examples: presumptive *mut7 mut1* double mutants were crossed to *mut1* testers for *mut1* allelism testing of the former. Presumptive *mut1 mut8* strains were crossed to *mut8* testers to test for the presence of *mut8* in the former. The presence of *mut8* homozygotes in the latter situation was confirmed for *his1-1* homozygous strains by using 3 μg/ml (3γ) limiting histidine Lassie test plates, instead of the usual 1 μg/ml (1γ) histidine (see Media). The results of these complementation tests are shown in the Appendix, Table A2.

Table 1: Strains used to construct stocks for this study

Haploid Strain	Genotype	Source
XV185-6A	α *MUT*$^+$, *his1-7, lys1-1, hom3-10, ade2-1, trp548, arg4-17*	S.K. Quah
XV379-20C	a *mut7-1, mut8-1, his5-2, lys1-1, ade2-1, trp5-48, arg4-17*	"
XV800-6C	a *mut1-1, his1-7, lys1-1, hom3-10, ade2-1*	"
XV354-2C	a *mut2-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
XV195-24C	a *mut3-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
XV357-12D	a *mut4-1, his5-2, lys1-1, ade2-1, trp5-48, arg4-17*	"
XV407-19D	a *mut5-1, his5-2, lys1-1, ade2-1, trp5-48, arg4-17*	"
XV374-28C	a *mut6-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
XV731-3D	a *mut8-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	"
XV396-1A	a *mut9-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	"
RO1-90A	α *mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	XV185-6A/XV379-20C
LA 2-15B	a *rad51-1, his1-7, lys1-1, ade2-1, trp5-48, leu*$^-$, *ura*$^-$	M. Mowat (1979)
EO 41-1A	a *rad52-1, his1-7, lys1-1, hom3-10, ade2-1, arg4-17, ade*$^-$, *ura*$^-$	L.E. Grant
KF179-15A	a *MUT*$^+$, *his1-1, lys1-1, trp5-48, cry1-1*	D.P. Morrison
KF178-44D	α *MUT*$^+$, *lys1-1, trp5-48*	"
RO400-10C	α *MUT*$^+$, *his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	RO1-90A/KF179-15A
YO600-14D	a *MUT*$^+$	S.K. Quah

TABLE 2: Strains used to construct *mut7* stocks free of other temperature sensitivities, to study spontaneous reversion at *his1-1*, and to examine spontaneous intragenic recombination at *his1*

Haploid Strains		Genotypes	Source
RO400-8A	a	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17, cry1-1*	RO1-90A/KF179-15A
8B	α	*mut7-1, his1-1, lys1-1, trp5-48*	"
8C	α	*MUT+, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
8D	a	*MUT+, his1-1, lys1-1, trp5-48, cry1-1*	"
RO401-5A	a	*MUT+, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17, cry1-1*	RO400-8A/KF178-44D
14B	α	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	"
15C	a	*mut7-1, his1-7, lys1-1, hom3-10, trp5-48*	"
RO402-6A	a	*MUT+, his1-1, lys1-1, trp5-48*	RO400-8B/KF179-15A
RO403-4A	α	*MUT+, his1-1, lys1-1, ade2-1, trp5-48*	RO400-8C/KF179-15A
RO405-2D	α	*MUT+, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	RO400-8A/XV731-10A*
RO415-8C	α	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	RO401-15C/RO400-10C
9C	a	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, cry1-1*	"
RO417-13A	a	*MUT+, his1-1, lys1-1, ade2-1, trp5-48, cry1-1*	XV185-6A/KF179-15A
RO428-1A	a	*MUT+, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, cry1-1*	RO415-8C/RO417-13A
B	a	*MUT+, his1-1, lys1-1, ade2-1, trp5-48*	"
C	α	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	"
2D	α	*MUT+, his1-7, lys1-1, ade2-1, hom3-10, trp5-48*	"
3C	a	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48,*	"
6B	a	*mut7-1, his1-1, lys1-1, ade2-1, trp5-48*	"
C	α	*mut7-1, his1-7, lys1-1, ade2-1, trp5-48, hom3-10*	"
7D	α	*mut7-1, his1-1, lys1-1, ade2-1, trp5-48*	"
15D	a	*MUT+, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	"

* see Table 7.

TABLE 2: (continued)

Haploid Strains		Genotypes	Source
RO88-2D	α	*MUT⁺, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	RO428-6B/RO78-3D
-4D	a	" " " " " " +	"
-8D	a	" " " " " " *arg4-17*	"
-14A	a	" " " " " " "	"
RO88-4C	α	*MUT⁺, his1-1, lys1-1, HOM⁺, ade2-1, trp5-48, arg4-17*	"
-5B	α	" " " " " " "	"
-9D	a	" " " " " " +	"
-14B	α	" " " " " " +	"
RO88-1D	α	*mut7-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
-7C	a	" " " " " " " +	"
RO88-1B	a	*mut7-1, his1-1, lys1-1, HOM⁺, ade2-1, trp5-48, ARG⁺*	"
-8B	α	" " " " " " " "	"
-9B	α	" " " " " " *arg4-17*	"
RO88-1C	a	*mut8-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
-2A	α	" " " " " " " "	"
-6A	a	" " " " " " " +	
RO88-1A	α	*mut8-1, his1-1, lys1-1, HOM⁺, ade2-1, trp5-48, ARG⁺*	"
-7A	a	" " " " " " " "	"
RO88-4B	α	*mut7-1, mut8-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	"
-9C	a	" " " " " " " " +	"
-10D	a	" " " " " " " " *arg4-17*	"
RO88-2B	a	*mut7-1, mut8-1, his1-1, lys1-1, HOM⁺, ade2-1, trp5-48, ARG⁺*	"
-4A	a	" " " " " " " " "	"
-6B	α	" " " " " " " " +	"
-8C	α	" " " " " " " " +	"

TABLE 2: (continued)

Diploid Strains	Source
RO400	RO1-90A/KF179-15A
RO401	RO400-8A/KF178-44D
RO402	RO400-8B/KF179-15A
RO403	RO400-8C/KF179-15A
RO404	RO400-8D/KF178-44D
RO405	RO400-8A/XV731-10A
RO406	RO400-8B/XV731-3D
RO407	RO400-8C/XV731-3D
RO408	RO400-8D/XV731-10A
RO409	RO400-8A/RO400-8D
RO410	RO400-8C/RO400-8D
RO411	RO400-8A/RO400-8B
RO413	RO401-14B/RO401-15C
RO415	RO401-15C/RO400-10C
RO416	RO401-15C/RO405-2D
RO417	XV185-6A/KF179-15A
RO422	RO415-8C/XV731-3D
RO423	RO415-9C/XV731-10A
RO424	RO415-8C/RO415-9C
RO425	RO402-6A/RO403-4A
RO426	RO402-6A/RO415-8C
RO427	RO417-13A/RO403-4A
RO428	RO417-13A/RO415-8C
RO429	RO403-4A/RO415-9C
RO516	RO428-6B/RO428-6C
RO517	RO400-10C/RO107-5C*

* see Table 5.

Table 3: Strains used to construct mutator stocks (other than *mut7*) capable of growth at 36°C

Haploid Strains	Genotype	Source
RO61-3C	a *mut1-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	XV800-6C/XV185-6A
RO62-2A	a *mut2-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV354-2C/ "
RO63-5C	a *mut3-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV195-24C/ "
RO64-3D	a *mut4-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV357-12D/ "
RO65-5A	a *mut5-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV4Q7-19D/ "
RO66-6B	a *mut6-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV374-28C/ "
RO69-2D	a *mut9-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	XV396-1A/ "

Diploids	Pertinent Genotype	Source
RO71	*mut/+, his1-7/his1-7,lys1-1/lys1-1*	RO61-3C/RO400-10C
RO72		RO62-2A/ "
RO73		RO63-5C/ "
RO74		RO64-3D/ "
RO75		RO65-5A/ "
RO76		RO66-6B/ "
RO78		XV731-3D/ "
RO79		RO69-2D/ "
RO91	*rad/+, his1-7/his1-7,lys1-1/lys1-1*	LZ2-15B/ "
RO92		LZ41-1A/ "

TABLE 4: Strains used to construct stocks for allelism testing of *mut* and *rad* loci with *mut7*, and for generating double mutant mutator spores (eg. *mut1mut7*)

Haploid Strains	Genotype	Source
RO71-7C	α *mut1-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	See Table 3
RO72-1C	α *mut2-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO73-5D	α *mut3-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO74-3A	α *mut4-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO75-4B	α *mut5-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO76-6D	α *mut6-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO78-3D	α *mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO79-8A	α *mut9-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO85-2B	a *mut5-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	RO75-4B/RO428-6B
RO85-2C	α *mut7-1,his1-1,lys1-1,ade2-1,trp5-48,arg4-17*	"
RO85-17B	a *mut5-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO85-17D	α *mut7-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO88-13C	a *mut7-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	RO78-3D/RO428-6B
RO89-2D	α *mut9-1,his1-1,lys1-1,ade2-1,trp5-48*	RO79-8A/RO428-6B
RO89-6C	α *mut9-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	"
RO91-1C	α *rad51-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	See Table 3
RO92-6B	α *rad52-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO92-9C	α *rad52-1,his1-7,lys1-1,hom3-10,ade2-1,arg4-17*	"
RO92-9D	α *rad52-1,his1-7,lys1-1,hom3-10,ade2-1,arg4-17*	"
RO106-4C	α *mut6-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	See Table 5

TABLE 4: (continued)

Diploid Strains	Source
RO81	RO71-7C/RO428-6B
RO82	RO72-1C/ "
RO83	RO73-5D/ "
RO84	RO74-3A/ "
RO85	RO75-4B/ "
RO86	RO76-6D/ "
RO88	RO78-3D/ "
RO89	RO79-8A/ "
RO93	RO91-1C/ "
RO94	RO92-6B/ "
RO504	RO85-2B/RO85-2C
RO505	RO85-17B/RO85-17D
RO506	RO106-4C/RO428-6B
RO507	RO89-2D/RO88-13C
RO508	RO89-6C/RO428-6B
RO510	RO92-9D/ "
RO511	RO92-9C/ "
RO518	RO92-6B/ "
RO519	RO75-4B/ "
RO520	RO75-4B/RO428-6B^r
RO521	RO75-4B/RO428-6B
RO522	RO75-4B/RO428-6B^r
RO523	RO75-4B/RO428-6B
RO524	RO75-4B/RO428-6B^r
RO525	RO75-4B/RO428-6B
RO526	RO75-4B/RO428-6B^r
RO99	RO400-10C/RO428-6B
RO100	RO88-2D/RO428-6B

TABLE 5: Strains used to construct stocks for allelism testing of *mut* and *rad* loci with *mut8*, and for generating double and/or triple mutant mutator spores (eg. *mut5mut7mut8*)

Haploid Strains	Genotype	Source
RO81-2C	α *mut1-1,his1-1,lys1-1,ade2-1,trp5-48*	See Table 4
RO82-6D	α *mut2-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO83-6C	α *mut3-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO84-4C	α *mut4-1,his1-1,lys1-1,ade2-1,trp5-48,arg4-17*	"
RO85-17A	α *mut5-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO86-21C	α *mut6-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO88-1A	α *mut8-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO88-1C	a *mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO88-4C	α *MUT$^+$,his1-1,lys1-1,ade2-1,trp5-48,arg4-17*	"
RO88-9C	a *mut7-1,mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	"
RO89-2D	α *mut9-1,his1-1,lys1-1,ade2-1,trp5-48*	"
RO89-6C	α *mut9-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	"
RO94-2A	a *rad52-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	"
RO104-5A	a *mut8-1,his1-1,lys1-1,ade2-1,trp5-48,arg4-17*	RO84-4C/RO88-1C
RO105-2A	α *mut5-1,mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	RO85-17A/ "
RO106-4C	α *mut6-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	RO86-21C/ "
RO107-5C	a *MUT$^+$,his1-1,lys1-1,ade2-1,trp5-48*	RO88-4C/ "
RO108-1C	a *mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48*	RO88-1A/ "
RO112-1B	α *mut5-1,mut7-1,mut8-1,his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*	RO88-9C/RO105-2A
RO255-3A	a *MUT$^+$,his1-7*	See Table 6

TABLE 5: (continued)

Diploid Strains	Source
RO101	RO81-2C/RO88-1C
RO102	RO82-6D/ "
RO103	RO83-6C/ "
RO104	RO84-4C/ "
RO105	RO85-17A/ "
RO106	RO86-21C/ "
RO107	RO88-4C/ "
RO108	RO88-1A/ "
RO109	RO89-2D/ "
RO111	RO94-2A/RO88-1A
RO112	RO88-9C/RO105-2A
RO113	RO112-1B/RO255-3A
RO118	RO112-1B/RO107-5C
RO512	RO106-4C/RO104-5A
RO514	RO89-6C/RO108-1C

TABLE 6: Strains used to construct stocks for studying forward mutation caused by *mut7* and/or *mut8*

Haploid Strain	Genotype	Source
RO88-7B	α *mut7-1,mut8-1,his1-7,lys1-1,ade2-1,trp5-48,arg4-17*	RO78-3D/RO428-6B
RO250-7A	α *mut7-1,mut8-1,his1-7*	RO88-7B/YO600-14D
RO252-1C	α *mut8-1,his1-7*	RO250-7A/YO600-14D
RO252-3B	a *mut7-1,his1-7*	"
RO255-1C	α *mut8-1,his1-7*	RO252-1C/RO252-3B
RO255-3B	α *mut7-1,mut8-1,his1-7*	"
RO255-3C	α MUT^+,*his1-7*	"
RO255-4A	α *mut7-1,his1-7*	"

TABLE 7: Genotype 'tester' strains employed in this study

Haploid Strains		Genotype	Locus Tested for	Selection	Source
XV185-14C	a	*MUT*$^+$, *his1-7*, *lys1-1*, *hom3-10*, *ade2-1*, *trp5-48*, *arg4-17*	a/α	histidine	S.K.Quah
-6A	α	" " " " " " "	"	"	"
XV185-6D	a	*MUT*$^+$, *his5-2*, *lys1-1*, *ade2-1*, *trp5-48*	a/α	histidine	S.K.Quah
-4A	α	" " " " "	"	"	"
X464-20C	a	*MUT*$^+$, *his2*, *ade1*, *trp1*, *leu1*, *gal1*, *mal*	a/α	histidine	S.K.Quah
-1A	α	" " " " " " "	"	"	"
LZ13-2C	a	*MUT*$^+$, *his1-1*, *lys1-1*, *ade1*	*his1-1*	lys or ade	L.Savage
-1A	α	" " " "	"	"	"
RO122-2C	a	*MUT*$^+$, *his1-7*, *lys2*, *hom3-10*, *ade2-1*	*his1-7*	lys or ade	G577-1A*
-1C	α	" " " " " *trp5-48*, *arg4-17*	"	"	RO400-10C
XV731-3D	a	*mut8-1*, *his1-7*, *lys1-1*, *hom3-10*, *ade2-1*, *trp5-48*, *arg*$^+$	*mut8*	zygotes	S.K.Quah
-10A	α	" " " " " " , *arg4-17*	"	dissected	"
RO105-2B	a	*MUT*$^+$, *his1-1*, *lys1-1*, *HOM*$^+$, *ade2-1*, *trp5-48*	Control	"	See Table 5
-1A	a	" , *his1-7*, " , *hom3-10*, *ade2-1*, *trp5-48*	Strain		

* from R.C.von Borstel, who obtained G577-1A from J. Game.

TABLE 7: (continued)

Haploid Strains		Genotype	Locus Tested for	Selection	Source
RO71-1D	a	*mut1-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	*mut1*	Zygotes dissected	see Table 3
-7C	α	see Table 4			
RO72-6D	a	*mut2-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48*	*mut2*	"	"
-1C	α	see Table 4			
RO73-2A	a	*mut3-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	*mut3*	"	"
-5D	α	see Table 4			
RO74-1B	a	*mut4-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	*mut4*	"	"
-3A	α	see Table 4			
RO75-1B	a	*mut5-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	*mut5*	"	"
-4B	α	see Table 4			
RO76-1C	a	*mut6-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	*mut6*	"	"
-6D	α	see Table 4			
RO78-8D	a	*mut8-1, his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*	*mut8*	"	"
-3D	α	see Table 4			
RO69-2D	a	see Table 3	*mut9*	"	"
RO79-8A	α	see Table 4			
RO88-7A	a	*mut8-1, his1-1, lys1-1, ade2-1, trp5-48, hom3-10*	*mut8*	"	see Table 4
-1A	α	*mut8-1, his1-1, lys1-1, ade2-1, trp5-48*			

TABLE 8: Genotypes of designated double mutator mutant strains crossed to *mut* tester strains for confirmation of the presence of particular *mut* loci by complementation tests

Haploid Strain	Genotypes	
RO81-2Aa	*mut1-1,mut7-1*	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
3Cα	" "	" " " " "
4Cα	" "	" " " " "
5Cα	" "	*his1-1,lys1-1,ade2-1,trp5-48*
6Aα	" "	" " " "
7Ba	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
10Ba	" "	*his1-1,lys1-1,ade2-1,trp5-48*
11Aα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
RO101-1Ba	*mut1-1,mut8-1*	*his1-1,lys1-1,ade2-1,trp5-48*
2Da	" "	*his1-1,lys1-1,ade2-1,trp5-48,arg4-17*
7Cα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*
8Ba	" "	" " " " " "
10Bα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
RO82-1Bα	*mut2-1,mut7-1*	*his1-1,lys1-1,ade2-1,trp5-48,arg4-17*
2Ca	" "	*his1-1,lys1-1,ade2-1,trp5-48*
3Ca	" "	*his1-1,lys1-1,ade2-1,trp5-48,arg4-17*
5Aa	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
6Ba	" "	*his1-1,lys1-1,ade2-1,trp5-48*
8Bα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
9Cα	" "	*his1-1,lys1-1,ade2-1,trp5-48,arg4-17*
10Bα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
RO102-1Bα	*mut2-1,mut8-1*	*his1-1,lys1-1,ade2-1,trp5-48,arg4-17*
2Bα	" "	" " " " "
4Da	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*
6Aα	" "	*his1-1,lys1-1,ade2-1,trp5-48*
7Ba	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*
10Bα	" "	*his1-1,lys1-1,ade2-1,trp5-48*
RO83-1Bα	*mut3-1,mut7-1*	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
4Cα	" "	*his1-1,lys1-1,ade2-1,trp5-48*
6Aα	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*
9Bα	" "	" " " " "
10Aα	" "	" " " " "
RO103-1Aa	*mut3-1,mut8-1*	*his1-1,lys1-1,ade2-1,trp5-48*
2Bα	" "	" " " "
5Ba	" "	" " " "
6Ca	" "	*his1-1,lys1-1,hom3-10,ade2-1,trp5-48,arg4-17*
8Cα	" "	*his1-1,lys1-1,ade2-1,trp5-48*
10Aa	" "	*his1-7,lys1-1,hom3-10,ade2-1,trp5-48*

TABLE 8: (continued)

Haploid Strain	Genotypes
RO84-1Aα	*mut4-1, mut7-1 his1-1, lys1-1, ade2-1, trp5-48, arg4-17*
2Aα	" " " " " " "
3Dα	" " *his1-7, lys1-1, hom3-10, ade2-1, trp5-48*
5Cα	" " *his1-1, lys1-1, ade2-1, trp5-48, arg4-17*
8Aa	" " " " " " "
9Ca	" " " " " " "
10Bα	" " " " " " "
RO104-1Da	*mut4-1, mut7-1 his1-1, lys1-1, ade2-1, trp5-48, arg4-17*
4Aa	" " " " " " "
6Ba	" " " " " " "
8Bα	" " " " " " "
RO105-1Cα	*mut5-1, mut8-1 his1-1, lys1-1, ade2-1, trp5-48*
2Aα	" " *his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*
5Ca	" " *his1-1, lys1-1, ade2-1, trp5-48, arg4-17*
6Aa	" " *his1-1, lys1-1, ade2-1, trp5-48*
8Da	" " " " "
10Aa	" " *his1-7, lys1-1, ade2-1, hom3-10, trp5-48*
RO106-4Dα	*mut6-1, mut8-1 his1-1, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*
5Cα	" " " " " " " "
8Aα	" " *his1-7, lys1-1, hom3-10, ade2-1, trp5-48*
10Aα	" " " " " " "
RO512-1Cα	*mut6-1, mut8-1 his1-7, lys1-1, ade2-1, trp5-48, arg4-17*
2Ca	" " " " " " "
3Cα	" " *his1-7, lys1-1, hom3-10, ade2-1, trp5-48, arg4-17*
5Ba	" " *his1-1, lys1-1, ade2-1, trp5-48, arg4-17*
6Bα	" " " " " " "
10Aα	" " " " " " "

B. Media

All recipes are for solid media. For liquid YD or MC medium Bacto-agar was omitted. All liquid cultures were incubated with shaking.

YD	:1% Bacto-yeast extract, 2% Bacto-peptone, 2% Bacto-dextrose and 2% Bacto-agar in distilled water (600 ml of medium).
YG	:identical to YD, except that 3% glycerol replaces dextrose.
MC (Mortimer Complete; See von Borstel *et al.*, 1973)	:0.67% Bacto-yeast nitrogen base without amino acids, 2% dextrose and 2% Bacto-agar; 24 mg of each of adenine, uracil, argincine, histidine, lysine, tryptophan and methionine, 36 mg of Leucine and 420 mg of threonine in a total of 36 ml of stock solution per 1.2 liters of medium.
Omission media	:MC without one or more of the amino acid or base supplements. These are referred to as "-(abbreviation for supplement)"; for example "-his".
Minimal medium (min)	:MC without any amino acid or base supplements.
cry medium	:2 µm crytopleurine (Chemsea Pty) per liter of YD.
FS (sporulation) medium	:1% potassium acetate, 0.1% Bacto-dextrose, 0.25% Bacto-yeast extract, 2% Bacto-agar, with amino acids and bases as in MC, in distilled water.

Tetrad Dissection medium	:YD with 3% Bacto-agar.
Lassie test media	:MC with reduced histidine or lysine to limit growth in histidine or lysine requiring strains, respectively, as follows
24 mg lysine ("20γ lys")	:identical to MC; lysine limits growth.
6 mg lysine ("5γ lys")	:lysine limits growth. Unless otherwise noted "limiting lysine" refers to 5γ lysine.
1.2 mg histidine ("1γ his")	:histidine limits growth. Unless noted, "limiting histidine" refers to 1γ histidine.
3.6 mg histidine ("3γ his") 72 mg lysine	:histidine limits growth. This was used to test spontaneous reversion of the *his1-1* allele in presumptive *mut8/mut8*, *his1-1/his1-1* strains (see Appendix, Table A2).
Buffer	:0.2 M K_2PO_4 buffer (monobasic) was utilized for all experiments and all handling of stocks, except where noted.

C. Handling of Stocks

All strains were incubated at 26°C unless otherwise noted.

1. Haploids

Stocks were maintained as haploids, and were stored on YD medium at 4°C. Strains to be used were streaked or plated onto YD medium for single colonies and incubated at 26°C for 3-4 days. Three clones were generally picked per strain and transferred in part to two YD master plates. The remainder of each colony was then suspended in buffer at 1-5 x 10^6 cells/ml, to assay for spontaneous mutation frequencies (Lassie tests; see below) and for mutagen sensitivities (spot tests; see below). Following 1-2 days incubation at 26°C, one YD master plate was replica-plated to omission media to test strains for auxotrophic markers and mating type, to YG medium for the detection of petite (ρ^-) isolates, and to three YD plates. One YD replicate was γ irradiated (40 krad; see below) and incubated at 26°C for 2-3 days, to allow the detection of γ-sensitive isolates. The second YD plate was incubated at 34°C (another YD replicate was also incubated at 36°C in the latter part of the study) for the detection of temperature-sensitive isolates. The third YD replica served as a control for the other two. The other YD master plate was refrigerated for future use, or was used as a second master plate when testing for mating type (see below).

2. Mating

Normally, to construct diploids, log-phase cells of opposite mating type were streaked from YD medium and mixed together thoroughly on a fresh YD plate. After allowing 4-6 hours at 26°C for conjugation, zygotes (with buds centrally located) were removed to a new location on the plate by use of a de Fonbrune micromanipulator The YD plate was incubated for 2-3 days at 26°C to allow the zygotes to form colonies.

Selected or forced matings were performed several times in this study. Two types will be noted here.

Rapid method for mating-type determination: those strains bearing *his1* were tested for mating type alleles by replica-plating. Two -his plates were seeded, each with one of a fresh, "a" or "α", *his5* tester strain (Table 7), shortly before conducting the replica-plating mentioned above. The seeding was most easily accomplished by suspending the tester strains separately in buffer at $\sim 10^7$ cells/ml, then spreading 0.5 ml of each suspension completely over separate -his plates. After the seeded plates dried, each was imprinted with the strains to be tested (using separate velvets and different master plates from the replica-plating series). Only strains complementing both for mating-type and for *his* genes mated and grew on the seeded -his plates. Plates were scored after three days for growth. This technique was also used to confirm a/α diploid phenotypes. Since the latter won't mate with haploid testers, complementation of the different *his* loci could not occur. The test may be used as a rapid screen for diploid mating type alterations (e.g. a/α versus a/a or a).

The second type of forced-mating was a procedure adapted from that of Savage (1979). It was used to distinguish between *his1-7* and *his1-1*-bearing haploids, by force-mating the (*lys1,ade2*) strain bearing the unknown allele to *his1* tester strains (see Tables 55 and 56). The *his1-1* tester strains bore *lys1* and *ade1*, while the *his1-7* tester strains bore *lys2* and *ade2* (Table 7). Forced-mating occurred on -ade or on -lys medium. Presumptive diploids were restreaked to -ade or to -lys medium, tested for diploidy as described above, and tested for *his1* allelism by assaying for U.V.-induced (∿13 joules/m^2; see below) recombination at *his1*. Recombinants were observed as large numbers of HIS^+ prototrophic clones arising in small streaks of *his1* heteroallelic diploid cells on -his medium, 2-3 days after U.V. irradiation. Homoallelic *his1* diploids produced few or no prototrophic clones per streak. In some cases, the U.V. results were confirmed by testing for enhanced $HIS1^+$ prototrophy using histidine Lassie tests (see Results).

3. Diploids and Sporulation

Diploid strains isolated as described in the previous section were tested in a manner identical to that described for haploids, with the following alteration. As part of the treatment of single clones, a small quantity of cells was streaked onto FS medium, and allowed to sporulate at 26°C for 5-7 days. Greater than 10% sporulation was observed in all strains, including *rad* heterozygous diploids, as determined by ascus formation.

4. Tetrad Dissection and Germination of Spores

Sporulated diploids were streaked from FS medium and suspended in 0.5 ml of glusulase (Endo), previously diluted 1/10. Following 20 minutes incubation at 26°C the ascus walls were digested sufficiently to allow tetrad dissection. The suspension was then diluted by the addition of 3 ml of buffer and refrigerated until used. Tetrads were dissected by micromanipulation (de Fonbrune) of spores onto an agar slab, normally within one week of the glusulase treatment. These were incubated for 3-5 days at 26°C (20°C in the latter part of the study) to allow germination and colony formation to occur. Each spore colony was then analysed as was described for haploid strains.

D. Measurements of Repair Parameters

1. Spontaneous Mutation and Mutator Phenotype Assay:

The Lassie Test

This test has been described and compared with the 1000 compartment fluctuation test by von Borstel (1978). It yields a mutation frequency of "revertants per Lassie test plate". For this assay, yeast requiring histidine and/or lysine was seeded onto medium where either histidine or lysine limited growth. The same number of cells from the same isolate was plated to -his or -lys medium as was plated on limiting histidine or lysine medium, respectively. Pre-existing and other spontaneous revertants to prototrophy for the limiting amino acid continue to divide after that supplement is exhausted, and form colonies. Unreverted cells

form a background lawn on the agar medium. Prototrophic clones which appeared on the omission plates were considered to be derived from pre-existing revertants. These clones were subtracted from those appearing on the respective limiting medium to obtain the Lassie score. A mutator yeast strain would be expected to produce more prototrophic clones on limiting medium and therefore to have an enhanced Lassie score.

The alleles used to test spontaneous reversion for histidine and lysine auxotrophs were normally *his1-7* and *lys1-1*, respectively. Revertant clones on each limiting medium were of two types. Crossfeeders comprised roughly 40% of *his1-7* revertants (and about 80% of *his1-1* revertants, in agreement with Fogel *et al.*, 1978), as detected by a halo of increased growth around the revertant clone. These prototrophs were usually included in the total histidine Lassie score. Mutations which suppressed the ochre allele *lys1-1* formed white colonies on limiting lysine medium, and comprised approximately 90% of the total numbers of revertants. Locus revertants of *lys1-1* comprised the remaining revertant colonies formed, and were often detectable as red prototrophic colonies (Schuller and von Borstel, 1973).

When describing revertants of these auxotrophs, numbers of pre-existing prototrophs are generally shown in parentheses. Where "jackpots" (relatively large numbers of pre-existing revertants) exceeded 75% of the revertants observed on limiting medium, the total number of revertants on limiting medium was tabulated, instead of the usual "total revertants minus pre-existing revertants". Alternatively, the abbreviation "JP" was substituted for the Lassie score.

All strains were tested routinely for mutator activity using the Lassie test. Cells were pre-grown and then suspended in buffer as described in section C1. Equal amounts of cell suspension were then pipetted to -his, limiting histidine, -lys and limiting lysine medium (0.5 or 0.33 ml/plate) and incubated at 26°C for six days. For 1γ his, 3γ his, and 5γ lys plates, this incubation time was normally sufficient to allow the expression of more than 90% of the revertants seen after 14 days' incubation, except where noted. Higher limiting concentrations of supplement necessitated longer incubation time, and sometimes did not result in proportionately greater numbers of revertants (data not shown).

Prototrophic colonies were counted with a Biotran II colony counter (New Brunswick Scientific Co., Inc.) or by hand if there were fewer than 30 estimated colonies per plate. The colony counter allowed a constant area on each plate to be scanned for clones. When the number of unreverted cells per area scanned was estimated, the Lassie test could be converted into an assay for spontaneous mutation *rate* as follows: after revertant colonies were counted, an agar plug 39.6 mm^2 in surface area bearing unreverted cells were removed from the limiting medium plates into one ml of buffer. The number of unreverted cells/ml (or/plug), N, was determined by hemocytometer count. The area screened by the colony counter was 4730 mm^2; the ratio of plate area screened to plug area counted was 119.4. Hence the spontaneous mutation rate, M, can be calculated from Lassie scores by

$$M = \frac{m - m_b}{2N \times 119.4} = \text{mutation rate per cell per division}$$

where 'm' is the number of revertants observed on limiting medium, "m_b" is the number of pre-existing revertants, and the factor of two accounts for the presence of twice as many cells (in a stationary phase culture) as there were cell divisions.

For one experiment a plug of a different size was used (Table 99); in this case the ratio of plate area screened/plug area counted was 60.2.

2. "Mini-fluctuation" Tests

Five-tube fluctuation tests were employed for the studies of forward mutation, and for the isolation of *mut7* revertants. Five log phase colonies were picked from YD medium, suspended in 5 mls of liquid YD and incubated with shaking at 26°C for four more days (one clone was suspended per tube). The growth of each clone was considered to be from one cell not mutated for the trait(s) under consideration. Hence the median number of mutants per plate could be directly transformed (see Lea and Coulson, 1949, or von Borstel, 1978) to mutation rates per cell per generation, without having to consider initial cell or initial revertant numbers, by using the method of the median.

Revertants of the temperature sensitivity in *mut7* strains were isolated by plating 0.5 ml of the stationary phase *mut7* cells from each tube (washed twice in buffer) onto YD plates at $\sim 10^7$ cells/ml. The plates were incubated for three days at 34°C for the RO1-90A strain, or at 36°C for RO428-6B and 6C. Temperature-resistant clones were restreaked onto fresh YD and retested at 34°C or 36°C. Only a few such *mut7* revertants were isolated from these strains (but see Table 17).

Auxotrophic mutants were isolated as follows. Stationary phase cells bearing only one auxotrophic marker, *his1-7*, and *mut7 mut8*, *mut7*, *mut8* or *MUT*$^+$ alleles were washed twice in buffer and adjusted to ~2 x 10^7 cells/ml. Appropriate dilutions were made to 2 x 10^6 or to 2 x 10^2 cells/ml. Aliquots of the 10^7 concentrations were plated to -his medium and to YD medium (two plates each per tube) to screen for *his1-7* and *mut7* reversion. 1 ml of cells from the 10^6 concentration were used for Lassie-testing the strains (two 1γ his plates, one -his plate per tube) to ascertain mutator phenotypes for each tube. Aliquots from the 10^2 concentrations were plated to YD medium (five plates per tube) to screen for viable cells from each strain. These YD plates were incubated for four days at 26°C, then replica plated to minimal medium which was supplemented only with histidine (min + his), to an altered MC medium containing 20 γ tyrosine (tyr) and isoleucine (ileu) in addition to the usual supplements, to YG medium for the detection of petite colonies, and to YD plates, which were γ -irradiated to screen for *rad* clones. Clones which failed to grow on the min + his replica plates were then tested twice on media containing histidine plus one (or two) of the eleven supplements present in the altered MC medium, to determine the nature of the auxotrophy.

The -his and YD plates were incubated at 26°C and 36°C for six days, respectively, and then scored for revertants.

3. Spontaneous Reversion of *his1-7* During and After Log Phase Growth

For these experiments, one YD culture (∿200 ml/flask) was used per strain tested, and only *his1-7* was screened for reversion. Aliquots of cells were removed at intervals during and after log phase growth of the culture, washed twice in buffer, and adjusted to between 10^6 and 10^7 cells/ml. Where viability was scored, further dilutions were made. 0.5 ml aliquots of the adjusted suspension were then plated to -his medium; the plates were incubated at 26°C and scored after three days (and after six days in some cases).

Rapid assay for stationary phase mutation expression: this assay required continuous subculturing of log phase (*mut8*) cells into test tubes containing fresh liquid YD. Subculturing must be initiated at less than 10^7 cells/ml. The old tube plus YD plus cells was then allowed to incubate for three days. The original culture was sampled for *his1-7* reversion at time zero, while the log phase subculture and the original culture were tested after three days' incubation at 26°C, for differences in revertant frequency per tube or per plate.

Residual growth of cells on omission medium was scored as reported by Magni and von Borstel (1962).

4. Assays for Sensitivities to Mutagens

Ultraviolet (UV) light irradiation: for induced *his1* recombination cells were streaked on YD plates, and exposed to UV light for 30 seconds, in the dark. The plates were then incubated

in the dark at 26°C for three days. The UV source was a low pressure mercury vapour lamp (Sylvania G03T8); the dose rate was 42 ergs mm^{-2} sec^{-1}, as determined by a Latarjet dosimeter.

Gamma ray (γ) irradiation: Strains were routinely spot tested or replica-plate tested for sensitivity to γ irradiation. The radiation source was ^{60}Co in a Gammacell 200 (A.E.C., Ltd.,). The dose rate was two krad./min. Twenty minutes of irradiation resulted in confluent growth on spots or replicates (on YD plates) of *RAD*$^+$ strains, but resulted in no growth in *rad* strains, except for occasional colonies per spot.

Methyl methanesulfonate (MMS) treatment of Growing Cells: Strains to be tested were spotted at $\sim 10^6$ cells/ml to YD plates containing 0.035% or 0.07% (V/V) MMS (see Prakash and Prakash, 1977). Viability was scored after two and after three days.

E. Diphenylamine Determination of DNA Content

The procedure of Roth (1974) was adapted for haploid cells. 10 ml samples were removed from liquid YD culture (initiated as described in section D3) starting at $\sim 10^7$ cells/ml, washed twice and adjusted to $\sim 10^8$ cells/ml. 0.01 ml of cell suspension from each sample was suspended into 1.0 ml of a 3% formalin solution for cell counts. The remainder was used for the diphenylamine reaction; the yield of DNA per ΔOD unit (595 nm - 650 nm readings) was 122 µgm (of calf-thymus DNA), over the range of OD readings used in the

experiments shown. All growth experiments involving *mut7* strains were performed essentially as in section D3, except that cultures were split into two subcultures after innoculation. One subculture was incubated at 26°C, the other at 36°C. Reversion tests were not normally performed in these studies.

F. Isolation of ρ^- Petite Strains

Haploid strains RO428-6C and RO400-10C were grown in liquid YD containing 10 µg/ml ethidium bromide for two subcultures (Goldring *et al.*, 1970). Survivors of this treatment failed to grow or produce ρ^+ revertants when plated to YG medium. Mitochondrial DNA was not detected in isolates from either strain when these were stained with 4^1, 6-diamidino, 2-phenylindole (DAPI, Serva), following the procedure described by Williamson and Fennel (1975).

RESULTS

A. Phenotypes of Strains bearing *mut7* and/or *mut8*

1. Mutator Phenotype

a. Mutator Activity for *lys1-1* or *his1-7* Reversion

Previous studies in this laboratory of spontaneous mutation in *mut* or *rad* mutants of yeast have generally relied upon data from the 1000-compartment fluctuation (or box) test (von Borstel, 1978). This study employed the Lassie test, which made it possible for large numbers of strains to be screened for differences in *his1-7* and *lys1-1* spontaneous reversion scores. With this assay, it is possible to count independently arising revertants, unlike most fluctuation tests. Furthermore, two types of revertants may often be screened for on one lysine Lassie plate; *lys1-1* suppressor revertant clones appear white, whereas *lys1-1* locus revertant clones are often red (note that in some strains this redness is not expressed, and hence *lys1-1* (red) locus revertants are not noted).

Table 9a gives mean Lassie test scores for spore clones from cross R088, which segregated for *mut7*, *mut8*, *mut7 mut8* and $MUT7^{+}$ $MUT8^{+}$ (which will be called MUT^{+}). *mut7* strains were determined by their *ts* phenotype (section A2), *mut8* strains by their relatively high lassie scores for *his1-7* or for *his1-1* reversion (see Tables 58, 59, 60 and 61). Both *lys1-1* locus and *lys1-1* total reversion frequencies have been noted, as well as *his1-7* reversion frequencies. Unless otherwise mentioned, *all* Lassie test scores in this study have been corrected for pre-existing revertants, by subtracting these (which appear on unsupplemented -his or -lys plates) from those counted on limiting medium.

From Tables 58-61, *lys1-1* locus (red) revertants are enhanced in *mut7* strains ($t=3.8$, $p<.01$) relative to *mut8* (or *MUT*$^+$) strains, whereas *his1-7* reversion is increased in *mut8* strains ($t=29$, $p<.01$) compared to *mut7* (or *MUT*$^+$) strains. Otherwise the two mutators are not particularly powerful by themselves. However, the presence of both *mut7* and *mut8* results in greater than additive (synergistic) increases in reversion frequency for both *lys1-1* and *his1-7*, in strains bearing both test alleles compared to those bearing either single mutator locus. Increases in *lys1-1* suppressor revertants (total Lassie test score minus locus revertants) appear to be additive [21(*mut8*) + 22(*mut7*) ~ 41(*mut7mut8*)]. The data shown in Table 9a are proportional to 1000-compartment fluctuation ('box') test data for related strains (Table 9c).

To ascertain that differences in reversion frequencies (per plate) were not due to different numbers of cells on the plate, the following protocol was used. Plugs of agar-plus-cells were taken from limiting medium after limited growth had occurred (revertant clones were avoided), and the number of cells per plug was counted by haemocytometer. There is no difference among strains for cells/plug (equals cells/plate divided by 119.4), as may be seen from Table 9b. The mutation rates (M_{lys} and M_{his} are the mutation rates to histidine and lysine independence, respectively) shown in this table compare well with those shown in Table 9c. The allele *his1-1*, whose reversion phenotypes in various strains are shown in Table 9b and Table 11, will be described in detail in Section A3, where it will be seen that it is also synergistically enhanced in *mut7 mut8* haploid or homozygous diploid strains.

To test for the suppression of *ade2-1*, *arg4-17* and *trp5-48*, prototrophic clones from lysine Lassie tests were picked and replica-plated to -ade and to -arg or -trp plates. The most frequent suppressors, Type I, suppress all three markers, as well as *lys1-1* (Gilmore and Mortimer, 1966). Table 10 gives the numbers of lysine locus or suppressor revertants for two concentrations of limiting lysine. Although none of the strains tested with 20γ lysine produced red revertant clones, the proportions of locus revertants was as predicted from Table 9c. The failure to produce a red coloration is probably due to one or more genetic factors, since the strains exhibiting red clones on 5γ lysine Lassie test plates were related to those tested at 20γ lysine. The difference is not due to the concentration of lysine, since many strains do produce red revertant clones on 20γ lysine Lassie plates, but it is affected by adenine concentration (see Schuller and von Borstel, 1972). What is evident is that all red revertant clones arising on limiting lysine in the *mut7*, *mut8* and *MUT*$^+$ strains are locus revertants, and that the latter are enchanced in the *mut7 mut8* strain. Hence, red *lys1-1* revertants are assumed to represent locus revertants on limiting lysine plates, where observed.

Table 11 summarizes the Lassie test scores for three homozygous test loci in diploid strains also homozygous for either *MUT*$^+$, *mut7*, *mut8* or *mut7 mut8*. The scores are similar to the haploid scores shown in Table 9. The data summarized in Table 11 may be seen in full in Tables 50 to 53.

TABLE 9a: Summary of haploid Lassie scores from cross RO88§

$$\left(\frac{mut7}{+}\ \frac{+}{mut8}\ \frac{+}{hom3\text{-}10}\ \frac{+}{his1\text{-}7}\ \frac{his1\text{-}1}{+}\ \frac{lys1\text{-}1}{lys1\text{-}1}\right)$$

his1-7 reversion	*MUT*$^+$	*mut7*	*mut8*	*mut7mut8*
Mean	11.6	26	136	659
Standard Deviation (S.D.)	4.6	1.0	71	145
Number (of strains tested	7	4	7	7
Standard Error (S.E.)	1.8	0.5	27	55
lys1-1 reversion*				
Mean	12.8(1)	35(13)	25(4)	96(55)
S.D.	4.1	12	5.2	23
Number tested	12	13	12	14
S.E.	1.2	3.5	1.6	6.3

§ see Table 58
* Numbers in parentheses are those of red (locus) revertants; see Table 61.

b: Mutation rates (M) of strains from cross RO88 in terms of (unreverted) cells per Lassie plate

Mutator	RO88 Strain	*his1* allele	Lysine Lassie score*	Cells/ plug* x 10^{-4}	M_{lys} x 10^{8}	Histidine Lassie score*	Cells/ plug* x 10^{-4}	M_{his} x 10^{8}
mut7	1D	1-7	26	138	7.9	39	162	10
	3D	1-1	22 (10)	165	5.6	2	104	0.8
mut8	1C	1-7	25	165	6.3	164	142	48
	3C	1-1	19 (9)	229	3.5	5	162	1.3
mut7, *mut8*	3B	1-7	103	106	40	865	122	296
	4B	"	77	146	22	752	139	226
MUT^{+}	(RO400) -10C¶	1-7	9 (1)	128	2.9	12	123§	4.1
	4C	1-1	11 (1)	103	4.5	0	139	<0.3

* Average of two determinations
¶ A *MUT*$^{+}$ 'control' or 'tester' strain
§ Average of four determinations

c: 'Box test' mutation rates of strains carrying *mut7*, *mut8* or the double mutant *mut7mut8* (S.K.Quah, unpublished data)

Mutator	XV Strain	Reversion Rates x 10^{8} at *lys1-1* suppressor	*lys1-1* locus	*lys1-1* Total	*his1-7*
mut7	732-2B	2.4	2.5	4.9	18.9
mut8	379-17C	3.9	3.4	6.3	43.2
	731-3D	2.1	2.9	5.0	77.5
mut7, *mut8*	379-28D	5.6	19.9	25.5	300.1
MUT^{+}	731-14A	1.3	0.4	1.7	6.1

TABLE 10: Locus revertants, and suppressors of *lys1-1* in *mut7*, *mut8* and *mut7mut8* strains arising on limiting lysine (Lassie) media

Mutator	RO strain	Number of clones screened	'Suppressors'		Locus revertants (LYS^+ade^-)**			Mean† lysine Lassie scores	Lysine/ml of medium
			LYS^+ADE^+ clones*	LYS^+ade^- clones*	Total clones	Red clones	Fraction of Total Revertants		
mut7mut8	1-12A	620	110	30	480	0	0.77	653	20µgm.
mut7	1-90A	211	115	2	104	0		109	"
	-90D	82	44	0	38	0		115	"
	Total	293	159	2	142	0	0.48		
mut8	1-90B	167	82	8	77	0		47	"
	-90C	167	91	2	74	0		43	"
	XV731-3D	53	23	0	30	0		47	"
	-10A	120	78	2	40	0		48	"
	Total	457	274	12	221	0	0.48		
MUT^+	1-12B	46	36	0	10	0	0.22	46	"
mut7	428-6B	45	28	4	13	13	0.29	42	5µgm.
mut7/*mut7*	516	112	56	13	43	43	0.38	41	"
mut8	108-1C	75	41	21	13	10	0.17	30	"
MUT^+	400-10C	21	15	5	1	0		9	"
	107-5C	34	23	7	4	1		15	"
	Total	55	38	12	5	1	0.09		

* These clones also had at least one other nonsense marker suppressed (*trp5-48* or *arg4-17*)
** Other markers (*trp5-48* and/or *arg4-17*) tested were not suppressed in these clones.
† At least two determinations/strain; both suppressor and locus revertants are included

TABLE 11: Summary of Lassie scores for diploids derived from cross RO88 haploids§

his1-7/his1-7 reversion	*MUT*$^+$/*MUT*$^+$	*mut7/mut7*	*mut8/mut8*	*mut7mut8/mut7mut8*
Mean	37	48	173	968
S.D.*	7.9	4.9	22	10
Number tested	6	4	8	3
S.E.	3.3	2.5	7.5	6
his1-1/his1-1 reversion				
Mean	< 0.4	< 1.0	5.3	34
S.D.*	0.6		1.0	2.3
Number tested	8	4	4	4
S.E.	0.2		0.5	1.2
lys1-1/lys1-1 reversion				
Mean	15.5	32	25	76
S.D.*	4.3	9.0	3.4	14
Number tested	25	18	21	19
S.E.	0.9	2.2	0.8	3.2
lys1-1/lys1-1 locus (red) revertants				
Mean	1.4	11.9	3.5	44
S.D.*	0.6	2.6	1.5	6.3
Number tested	22	19	18	12
S.E.	0.2	0.6	0.4	1.8

§ See Tables 50,51,52 and 53.
* Only S.E.s are shown in subsequent summary Tables. Numbers of strains tested may be obtained from the (tetrad) analyses included with such summary Tables.

b. Forward mutation

Strains bearing *mut7* and *mut8* show synergistic enhancement of all types of spontaneous nuclear mutation, except *lys1-1* suppressor mutations, which are additively increased (Table 9). The fluctuation tests shown in Tables 12 and 13 indicate that the spontaneous forward mutation to auxotrophy in *mut7 mut8* strains greatly exceeds that shown in MUT^+ strains or strains bearing *mut7* or *mut8* individually. The single mutants show some increase over the MUT^+ strain.

A red ade^- mutant and a thr^-met^- clone failing to complement *hom-3* were among auxotrophs isolated from strain RO255-3B, which contains *mut7*, *mut8* and *his1-7*. Two other auxotrophs grew if either methionine *or* threonine was added to the medium. A red ade^- mutant was also isolated from the *mut8* strain.

The RO255 strains were screened for *rad* mutants in the same experiment. One γ -radiation sensitive and one UV-sensitive mutant were isolated in the double mutator, and one γ -radiation sensitive mutant was found in the *mut7* strain.

Data for several types of spontaneous mutation are summarized in Tables 14 and 15. Except where noted, the median number of mutants per ml. of cells plated was transformed appropriately (see Materials and Methods) using the method of the median (Lea and Colson, 1949), and divided by twice the *mean* viable cell count for all five tubes tested, to obtain a mutation rate per viable cell (observed colony) per generation. For comparison, *his1-7* and presumptive *mut7* (*ts*) reversion rates are included.

Estimates of plating efficiency (hemocytometer count versus viable cells) were made in the RO255 strains (Table 15). To minimize differences, budded cells were counted as one expected colony. The *mut7 mut8* haploid had a reduced number of observed, compared to expected colonies (means of 291/358; t=3.46, p<.025). None of the other three strains had significant reductions in plating efficiency. This result duplicated an earlier observation which had indicated that a *mut7 mut8* strain produced fewer total cells, fewer viable cells and lowered plating efficiency, compared with *MUT*$^+$, *mut7* or *mut8* strains, during and after growth in liquid YD medium (data not shown). It seems likely that this phenotype is common in *mut7 mut8* strains, since clones from such strains generally take longer to grow, and form smaller colonies on YD medium.

Reduced plating efficiency of a mutator mutant might be caused by one or both of the following: either lethal forward mutations are occurring at a sufficient rate to be detected, or existing repair systems are incapable of coping with lesions "spontaneously" introduced into nuclear DNA, resulting in failure of the cell to divide. The latter alone is unlikely becuase homozygous *mut7 mut8* diploids showed a large increase in 2:2 segregation for spore lethality when held in the diploid state for several weeks, compared with the same cross sporulated within a week of mating. This phenotype is also seen in *mut7* homozygotes. [spore viabilities for crosses are given in TableA3 in the Appendix]. Diploid *HIS*$^+$ clones picked from Lassie plates (as part of the experiment shown in

Tables 55 and 56) were sporulated necessarily after two weeks as diploids. Here too the *mut7* homozygote had large numbers of 2:2 segregations for non-germinating spores, compared to a MUT^+ homozygous strain sporulated at the same time. This type of spore lethality is generally considered to be due to recessive lethal mutations. Such mutations would result in lethal sectors and reduced plating efficiency in haploids, as was reported above.

Tables 14 and 15 also show spontaneous petite frequencies for the RO255 haploids. The large reduction seen in the *mut7* strain was also observed in other *mut7* stocks, including RO428-6B. A similar five-tube test showed that this strain had half the number of petites observed in the *mut7* revertant RO428-$6B^r$11 (However, the sister spore RO428-6C and its revertant had identical petite frequencies. In addition, the phenotype varies with growth conditions, such as phase of growth when sampled, and time in buffer after growth). Since nuclear petites contribute significantly to total petites isolated by various techniques (Sager 1972), and since *mut7 mut8* strains enhance nuclear mutation, it is possible that the larger numbers of petites observed in the *mut7 mut8* strain RO255-3B were due to nuclear mutation, but this possibility was not tested.

TABLE 12: Spontaneous appearance of auxotrophic clones in *MUT*$^+$ and *mut7mut8* strains

Strain	No. of tubes	his	lys	thr	met	thr & met	thr or met	ade	arg	trp	ura	tyr	leu	ileu	other	Total	No. of Clones tested	Auxotroph Frequency /cell
RO255-3C *MUT*$^+$	5	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4163	<.0003
RO255-3B *mut7mut8*	1	*	1	0	1	0	1	0	1	1	1	1	1	0	0	8	622	.013
	1	*	1	0	1	1	0	2	1	1	0	0	0	1	1	9	556	.016
	1	*	1	1	1	2	0	0	0	1	0	0	0	0	1	7	619	.011
	1	*	0	0	0	0	1	1	1	0	0	0	0	0	1	4	550	.007
	1	*	1	0	1	0	0	0	1	0	1	0	0	0	1	5	565	.009
	all 5	*	4	1	4	3	2	3	4	3	2	1	1	1	4	33	2912	.011
XV379-28D*** *mut7mut8*	1	*	*	*	*	*	*	*	*	*	0	1	1	**	1	3	1877	.002
XV379-20C*** *mut7mut8*	1	*	*	0	0	0	0	*	*	*	1	0	0	**	1	2	1452	.001
XV379-28A*** 20D*** *mut7mut8*	2	2	*	1	0	0	0	*	*	*	1	0	1	**	0	5	670	.007

* not tested, due to pre-existing auxotrophic marker.
** not tested
*** data from S.K.Quah (unpublished)

Table 13: Spontaneous appearance of auxotrophic clones in *mut7* and *mut8* strains

Strain	No. of tubes	Independently arising auxotrophs for: his	lys	thr	met	thr & met	thr or met	ade	arg	trp	ura	tyr	leu	ileu	other	Total	No. of Clones Tested	Auxotroph Frequency /cell
RO255-4A	1	*	0	0	1	0	0	0	0	0	0	0	0	0	0	2	977	.002
mut7	1	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	781	<.001
	1	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	757	<.001
	1	*	0	0	0	0	0	0	1	0	0	0	0	0	0	1	807	.001
	1	*	0	0	0	0	0	0	0	0	1	0	1	0	0	2	697	.003
	all 5	*	0	0	1	0	0	0	1	1	1	0	1	0	0	5	4019	.001
RO255-1C	1	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	905	<.001
mut8	1	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	709	<.001
	1	*	0	0	0	1	0	0	0	0	0	0	0	0	0	1	652	.002
	1	*	0	1	0	0	0	0	0	0	0	0	0	0	0	1	584	.002
	1	*	0	0	0	0	0	1	0	0	0	0	0	0	0	1	872	.001
	all 5	*	0	1	0	1	0	1	0	0	0	0	0	0	0	3	3722	.001

*not tested because of pre-existing auxotrophic marker.

TABLE 14: Forward and reverse mutation rates in *MUT*$^+$, *mut8*, *mut7* and *mut7mut8* strains

	Strain RO255-	Type of Mutation: Inviable Cells	Petites	*his1-7* revs.	*mut7* revs.	Auxotrophs
Median Score of mutants per ml. of cells plated	3C *MUT*$^+$	-	236	32	-	-
	1C *mut8*	-	116	364	-	1
	4A *mut7*	-	32	79	6	1
	3B *mut7, mut8*	71	62	356	228	6
Mean No. of viable* clones screened per ml.	3C *MUT*$^+$	519	833	555×10^5	-	833
	1C *mut8*	766	744	744×10^5	-	744
	4A *mut7*	418	804	402×10^5	402×10^5	804
	3B *mut7, mut8*	358	582	291×10^4	291×10^5	582
Mutation rate per viable * cell per generation	3C *MUT*$^+$	-	.028	8.3×10^{-8}	-	-
	1C *mut8*	-	.017	45.0×10^{-8}	-	.0006 **
	4A *mut7*	-	.006	23.5×10^{-8}	3.3×10^{-8}	.0006 **
	3B *mut7, mut8*	.024 (.20)†	.013	1.13×10^{-5} (7.18×10^{-6})§	77.6×10^{-8}	.0026 (.01)†

* For cell inviability, expected clones (hemocytometer count with budded cells counted as one cell) are used instead of viable clones

† calculation based on the rationale of Ogur *et al.* (1959)

§ " " " total cells, rather than viable cells

** " " " the P_0 estimation of Lea and Coulson (1949)

TABLE 15: Forward and reverse mutation in *HIT*$^+$, *mut7*, *mut8*, and *mut7mut8* strains: five-tube fluctuation tests

Strain 80255-	Total cells $x10^{-6}$	Fraction of cells budded	Expected colonies $x10^{-6}$	Observed colonies $x10^{-6}$**	Petite colonies $x10^{-6}$**	Existing *his* revertants $x10^{-1}$	*his*$^+$ clones arising on limiting histidine$^+$	*is* revertant colonies $x10^{-1}$
3C (*HIT*$^+$)	609	.16	525	542	175	3.2	33	
	580	.11	511	535	136	1.2	34	
	570	.13	506	559	145	1.7	36	
	617	.13	546	544	159	3.1	28	
	595	.17	510	596	157	3.5	46	
Means	594	.15	519	555	154	2.5	35	
1C (*mut8*)	945	.09	864	905	96	46.9	269	
	795	.08	734	709	120	36.4	279	
	868	.16	750	652	132	33.6	201	
	804	.13	712	584	103	32.0	285	
	894	.15	772	872	116	45.4	254	
Means	861	.12	766	744	113	38.9	258	
4A (*mut7*)	553	.27	456	489	14	7.9	114	.6
	524	.29	405	391	23	6.3	167	.6
	553	.21	457	379	21	6.6	145	.2
	511	.24	411	404	16	8.1	146	1.0
	478	.26	378	349	15	10.3	133	.7
Means	524	.25	418	402	18	7.8	147	.6
3B (*mut7, mut8*)	437	.27	343	311	27	356	2486	18.2
	480	.29	349	278	31	477	2689	37.3
	436	.31	334	309	32	340	2570	18.5
	463	.53	349	274	31	295	2445	22.8
	502	.20	417	383	26	395	2473	33.5
Means	458	.28	358	291	29	372	2533	26.1

Numbers are all per ml. of culture.
* Correction for pre-existing revertants (in the previous column)
** Numbers calculated based on YD plate counts and appropriate dilution factors

c. Expression of Revertants of *his1-7* in *mut8-1* Strains

Of the strains studied during the course of this work, those bearing *mut8* consistently produced large numbers of *his1-7* → *HIS*$^+$ prototrophs on -his plates as well as on plates with limiting histidine, when the strains were pregrown to stationary phase. When late log phase *mut8* cells were suspended in buffer and incubated at 26°C overnight before plating on -his medium, increases in the numbers of revertants per cell were noted, compared to the same suspension plated without prior incubation (data not shown). Further incubation in buffer resulted in no further increase in the number of revertants per cell. When two-day-old stationary phase *mut8* cells were tested similarly, there was no such increase.

Table 16 indicates that net residual growth of *mut8* strains was not responsible for the increased reversion, whether the cells were suspended in buffer or spread on -his plates. Cells from late log phase (two days on YD plates) show no residual growth on -his if previously washed and held in buffer or if suspended cells were directly transferred to minimal medium. The cells only doubled in number when transferred directly to -his plates.

To determine whether the buffer, or cell arrest itself was causing the enhanced spontaneous mutability, cells were incubated in liquid YD in log phase and then held in stationary phase for several days. Aliquots of this culture were removed at various times, washed twice and immediately plated on -his medium. The results for *mut8* are shown in Table 18 and those for *MUT*$^+$ in Table 17. The *mut8* strain

showed a fifty-fold increase in expressed revertants per cell between early log and early stationary phases, while the *MUT*$^+$ strain showed no detectable increase. The largest increase in *his1* prototrophs in the *mut8* strain occurred between one and two days in culture when total cells had stopped increasing and budded cells had reached stationary phase levels. Since time in buffer was constant and short for all samples, sensitivity of *mut8* cells to mutations induced by the buffer would have to be confined to the brief time when cells are entering stationary phase. It would appear, therefore, that the enhancement is a function of entry into stationary phase, and probably not due to the buffer. The data in Tables 17 and 18 also show that increased revertants are not due to their preferential viability in a dying culture, since viable cells (as measured by observed colonies) do not decrease significantly after stationary phase is reached.

Preferential growth of *mut8* histidine prototrophs might be responsible for the mutator activity of *mut8* when the cells enter stationary phase. However, the following three observations do not support this contention (note that at least seven cell divisions would have to occur *per prototroph* in stationary phase culture, to account for the increase).

(1) *mut8* strains are still mutators in the Lassie test, where most prototrophs arise independently and therefore give rise to single colonies. Preferential *HIS*$^+$ cell growth under these conditions may lead to enhanced prototroph clone size, but not to more clones.

(2). When *MUT*$^+$ cells were taken from a YD culture, incubated on -his medium for six days instead of the usual three, and counted after both three and six days, no large increases in *HIS*$^+$ revertants per cell were observed between counts (Table 19). This is true both for log and stationary phase *MUT*$^+$ cells. The *mut8* strain, however, showed large increases in *HIS*$^+$ revertants per cell between three and six days incubation on -his medium, but only in cells taken from log phase (Table 20:cf.0,6 and 18 hours after innoculation). No such increases were observed in aliquots of stationary phase *mut8* cells plated to -his medium. The simplest interpretation is that log phase *mut8* cells enter stationary phase on the -his plate, and then independently express their revertants (as in the Lassie test).

(3) Preferential growth of *mut8 HIS*$^+$ cells does not take place in log phase. Both Tables 19 and 20 (bottom row of data) show that *mut8* cells maintained in log phase by subculturing actually show a decrease in revertants per cell at 24 hours, compared to both the time 0 sample and to the 24 hour (early stationary phase) sample.

Histidine-feeder revertants did not cause large increases in total prototrophs in these experiments (due to crossfeeding). This can be seen from Tables 19 and 20. Neither *mut8* nor *MUT*$^+$ stationary phase levels of revertants increased between three and six days' incubation on -his plates, as would be expected if significant crossfeeding was occurring.

When *mut8* cells are maintained in log phase for three days (by subculturing), there is no enhancement in revertants per cell over the

zero time sample, while three day, stationary phase *mut8* cultures show the usual increase (Table 21). Interestingly enough, certain *MUT*$^+$ strains may show the "*mut8* effect" of enhanced spontaneous mutability upon entering stationary phase. Strain RO400-10C in Table 21 shows some stationary phase increase in revertants per cell, while RO105-1A, and XV185-6A (Tables 17,18,19 and 20) do not.

It should be emphasized that the "*mut8* effect" has only been noted for *his1-7* (and possibly *trp5-48*). Other loci, such has *lys1-1* or *hom3-10* do not revert at rates sufficient to detect the effect on Lassie tests or on replica plates. It is assumed that this effect was responsible for the enhanced numbers of histidine revertants observed (in *mut8* strains) on the -his medium used to detect pre-existing histidine prototrophs in histidine Lassie tests.

TABLE 16: Residual growth of *mut8* or *MUT*$^+$ cells on -his or minimal plates after two days incubation on YD

Strain	Hours after transfer	Plate	Number of cells in each group of cells on the plate 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total cell count	Total no. of groups	Average cells/ group	Residual Growth Index
XV731-3D**	1	-his	515	154	45	10	2	1	1										1021	728	1.40	
mut8	64	-his	209	250	166	65	54	35	25	8	7	5	4	1	1				2354	828	2.84	2.03
XV185-6A**	1	-his	604	155	68	15	4	3	0	0	0	1							1166	830	1.40	
mut$^+$	64	-his	192	285	148	73	45	26	19	12	4	4	7	2	1	0	1	5	2361	822	2.87	2.05
XV731-3D**	1	min.	570	164	56	18	6	2	0	1									1188	817	1.45	
mut8	64	min.	553	122	45	18	6	2	3										1096	822	1.47	1.01
XV185-6A**	1	min.	532	118	24	6													864	680	1.27	
mut$^+$	64	min	558	123	40	15	4	3	2										1028	743	1.38	1.09
XV731-3D*	1	-his	595	189	30	2	1												1019	797	1.27	
mut8	48	-his	514	221	51	13	6	0	0	1									1119	806	1.49	1.17
	72	-his	522	143	29	7	2	1											939	704	1.33	1.05
XV731-10A*	1	-his	758	165	16	5													1156	944	1.22	
mut8	48	-his	579	191	54	19	2	2											1221	847	1.44	1.18
XV185-6A*	1	-his	684	132	11	1	1												983	828	1.19	
mut$^+$	48	-his	545	123	20	14	3												920	708	1.31	1.10
	72	-his	575	125	33	9	1	1											977	746	1.31	1.10

** Cells were transferred directly from YD plates without washing. * Cells were washed twice, and held at room temperature for nine hours after growth on YD before plating to -his.

TABLE 17: *HIS*$^+$ prototrophs arising spontaneously in strain XV185-6A (*MUT*$^+$, *his1-7*) during growth in liquid YD medium

Time after Innoculation Hours	Days	Total* cells/ml. ($x10^{-6}$)	Fraction of cells budded	Observed colonies per ml.** ($x10^{-6}$)	*his*$^+$ Prototrophs† per ml.	per total cells ($x10^6$)	per viable cells ($x10^6$)	Total counted
0		2.82	0.73	1.60	0.3	0.1	0.2	1
2		3.60	0.79	2.43	3.8	1.0	1.5	3
12		202	0.56	151	12	0.1	0.1	1
24	1	396	0.22	346	300	0.8	0.9	6
	2	330	0.13	334	350	1.1	1.1	7
	3	343	0.11	431	450	1.3	1.0	9
	4	277	0.09	377	600	2.2	1.6	12
	5	369	0.08	419	400	1.1	0.9	8
	6	356	0.08	361	450	1.3	1.2	9
	8	350	0.10	373	450	1.3	1.2	9
	12	335	0.08	352	350	1.0	1.0	7

* A minimum of 900 cells were counted using a haemocytometer. Budded cells were counted as two or three cells, depending on the number of buds.

** A minimum of 150 clones on four YD plates were counted.

† Counted after three days incubation on -his plates.

TABLE 18: *HIS*$^+$ prototrophs arising spontaneously in strain XV731-3D (*mut8-1, his1-7*) during growth in liquid YD medium

Time after Innoculation Hours	Days	Total* cells/ml. ($x10^{-6}$)	Fraction of cells budded	Observed colonies per ml.** ($x10^{-6}$)	*his*$^+$ Prototrophs † per ml.	per total cells ($x10^6$)	per viable cell ($x10^6$)	Total Counted
0		2.08	0.71	1.14	6.	2.9	5.3	12
2		3.25	0.77	2.05	15	4.6	7.3	11
12		100	0.61	66.8	600	6.0	9.0	30
24	1	353	0.22	293	4600	13.0	15.7	91
	2	321	0.14	345	56500	176	164	1130
	3	315	0.09	322	62500	198	194	1249
	4	346	0.09	347	64100	185	185	1262
	5	366	0.09	361	48300	132	134	965
	6	336	0.11	332	47200	140	142	943
	8	351	0.09	340	43700	125	129	874
	12	317	0.06	381	42700	135	112	854

* A minimum of 900 cells were counted using a haemocytometer. Budded cells were counted as two or three cells, depending on the number of buds.

** A minimum of 150 clones on four YD plates were counted.

† Counted after three days incubation on -his plates.

TABLE 19: *HIS*$^+$ prototrophs arising spontaneously in strain RO400-10C (*MUT*$^+$, *his1-7*) grown in liquid YD medium (scored after three and six days incubation on -his plates).

Time after Innoculation Hours	Days	Total* cells/ml. ($x10^{-6}$)	Fraction of cells budded	*his*$^+$† after 3d on -his plates: per ml. of culture	per total cells ($x10^6$)	Total Counted	*his*$^+$† after 6d on -his plates: per ml. of culture	per total cells ($x10^6$)	Total Counted
0		0.81	0.64	0.13	0.16	10	0.13	0.16	10
6		7.0	0.64	0.38	0.05	3	0.75	0.11	6
18		319	0.38	42.5	0.13	17	72	0.23	29
24	1	438	0.17	45	0.10	18	78	0.18	31
	2	480	0.12	230	0.48	45	245	0.51	49
	3	477	0.12	210	0.44	42	245	0.51	49
	7	584	0.08	430	0.74	172	485	0.83	194
24 (cells maintained in log phase)		584	0.58	37	0.38	37	4.9	0.40	39

* A minimum of 900 cells were counted using a haemocytometer. Budded cells were counted as two or three cells, depending on the number of buds.

† Prototrophic clones.

TABLE 20: *HIS*$^+$ prototrophs arising spontaneously in strain RO78-3D (*mut8-1, his1-7*) grown in liquid YD medium (scored after three and six days incubation on -his plates)

Time after Innoculation Hours	Days	Total** cells/ml. ($x10^{-6}$)	Fraction of cells budded	*his*$^+$† after 3d on -his plates: per ml. of culture	per total cells ($x10^6$)	Total Gounted	*his*$^+$† after 6d on -his plates: per ml. of culture	per total cells $x10^6$)	Total Counted
0		0.98	0.57	1.35	1.38	108	16.7	17.1	1336
6		7.1	0.56	11.5	1.62	93	145	20.5	1160
18		213	0.28	1530	7.18	612	4340	20.4	1936
24	1	416	0.12	4300	10.3	1719	7710	18.5	3084
	2	509	0.08	54150	106	10829*	53280	105	10656*
	3	498	0.05	63030	126	12181*	60900	122	12180*
	7	473	0.04	42700	90.3	1707	56800	114	1988
24 (cells maintained in log phase)		7.5	0.54	5.8	0.76	46	16.0	2.13	128

** A minimum of 900 cells were counted using a haemocytometer. Budded cells were counted as two or three cells, depending on the number of buds.

† Prototrophic clones.

* Minimum estimates made using an electric colony counter.

TABLE 21: Timing of expression of *his1-7* reversions in *mut8* or *MUT*$^+$ strains (rapid assay results)*

Strain		*his1-7*→*his*$^+$ reversion frequency/10^6 cells in liquid YD at time 0 (log phase)		3 days (log phase)		3 days (stationary phase)	
RO400-10C	*mut*$^+$	0.7	(109)	0.3	(152)	5.6	(759)
		0.2	(20)	0.2	(14)	2.1	(81)
RO105-1A	*mut*$^+$	0.5	(20)	0.5	(29)	0.8	(31)
XV731-3D	*mut8*	7.1	(333)	6.6	(959)	141	(1640)
		4.7	(45)	2.7	(125)	251	(988)
RO78-8D	*mut8*	1.9	(133)	1.6	(116)	105	(629)
		2.0	(555)	4.8	(613)	270	(1189)

* Prototrophs were counted after three days incubation on -his plates. Actual numbers of prototrophs observed are given in parentheses.

2. Temperature - sensitivity of *mut7*

Crosses involving *mut7* have consistently co-segregated for mutator activity and inability to grow at 34°C, when crossed with strains capable of growth at this temperature. In spite of the small increase in mutator activity observed in *mut7* versus *MUT*$^+$ strains, the *ts* phenotype was found to co-segregate with higher lysine Lassie scores more than 95% of the time (see Appendix Table A4 and Figure A2, and also Tables 34,36,37,44 and 48). While histidine scores were more variable, these also tended to be higher in *ts* strains (Appendix Figure A2). The *ts* phenotype was found in *mut7mut8* strains and in *mut7/mut7* diploids (data not shown).

To ascertain the viability of cells bearing mutator loci *mut1* to *mut9*, or *MUT*$^+$ loci at different temperatures, approximately 10^6 stationary phase cells were spot-tested at a time for each strain (or about 10^2 cells from some strains were plated) on YD plates. These were incubated at 34°, 30°, 23° or 18°C for 3 - 4 days (the normal incubation temperature was 26°C). Strains designated *mut7* did not form colonies or otherwise grow at 34°C, but grew as well as *MUT*$^+$ strains at other temperatures. All of the other mutators segregated away from *ts* markers present in the original strains (see Appendix Table A1).

Three *ts* "terminal phenotypes" were observed microscopically in the stocks screened. Strains bearing *mut7* assumed a dumbbell morphology. In other *ts* strains cells became large singlets (greater than 2 - 3 times normal diameter) or mimicked the cell division cycle mutant, *cdc11* terminal phenotype (see Hartwell *et al.*, 1973).

Figure 1 and Tables 22, 23 and 24 show the cell arrest and loss of viability after 12 hours at 34°C seen in the *mut7* strain R01-90A, compared with the *MUT*$^+$ strain XVI85-6A. Both strains were growing logarithmically in MC medium at the time of the shift. The number of *his1-7* and *lys1-1* revertants per viable cell were noted, and appeared to increase in the *mut7* cells held at 34°C after nine hours. However, the numbers of revertants/plate were less than five, and the observation has not been re-tested.

Numbers of stationary phase cells did not exceed 5×10^7 cells/ml because MC medium usually limits cell growth at lower cell densities, due to lysine starvation.

a. Co-reversion of *mut7* phenotypes with the *ts* Phenotype

The cosegregation of the *ts* and mutator phenotypes did not rule out the possibility that *mut7* might be closely linked to a separate *ts* mutation. To do so, spontaneous revertants of the *ts* phenotype were selected in the *mut7* strains 401-90A. R0428-6B and R0428-6C, by plating $\sim 10^7$ cells to YD at 34° or 36°, and picking colonies growing at those temperatures. Since five-tube fluctuation tests were used, only one such colony per table was picked. These colonies were then screened, using the Lassie test for the *mut7*-mediated mutator activities discussed in Results, part 1. All but one (Table 25) had *MUT*$^+$ mutator activity on histidine and lysine. In addition, the MMS-sensitivity (Nasim and Brychcy, 1977) and enhanced spontaneous recombination (this thesis) observed in *mut7* strains also coreverted with the *ts* phenotype. These data are summarized in Table 25. All the revertants retained the same auxotrophies as were present in the *ts* parent, hence it is unlikely that these revertants were due to contamination.

Tables 26, 27 and 28 show segregants from crosses of *mut7-1*, and of the *ts* revertants *mut7-1-1* and *mut7-1-2* to a *mut8* strain. The typical *mut7 mut8*-mediated synergistic enhancement of reversion in histidine and lysine Lassie tests was noted for several segregants of the control cross (Table 26). Nothing of the sort was noted in any segregants from the cross of the revertant allele *mut7-1-11* to *mut8-1*. All other markers present in these crosses segregated normally. Similar results were observed for a similar cross involving the revertant strain R0428-6B (*mut7-1-11*: compare the data in Table 58 with those in Appendix Table A5).

The cross involving *mut7-1-2* segregated for high histidine Lassie test scores but not for lysine Lassie test scores (Table 28). I interpret this to be due either to an intragenic second-site revertant or to some type of linked modifier mutation. Class I suppressors do not affect *mut7*, since *mut7 SUP6* strains remain temperature sensitive (data not shown). Some support for the interpretation of a linked modifier mutation was found in the observation that white clones (*ade*$^-$, p^+) frequently segregated from *mut7* strains with the ability to grow marginally at 36°C. However, the *mut7-1-2* haploid strain was red in colour.

Some co-reverting phenotypes noted in Table 25 are dealt with elsewhere (see section B3 for lethality with *mut5* and section A3c for *mut7* mediated recombination enhancement). Two other phenotypes not mentioned in Table 25 appear to co-revert with the *ts* phenotype. These are: the doublet phenotype observed in *mut7* strains at 36°C, and the reduction in petite frequencies noted in section A1b. However, as noted, and as was the case with the *mut7 mut5* lethality,

the latter phenotype itself is variable in expression in certain *mut7* strains.

b. Terminal Phenotype of *mut7* Strains at 36°C

As previously noted, 90-98% of *mut7* (or *mut7/mut7*) log phase cells became large doublets (or occasionally triplets) within four hours of shifting the incubation temperature from 26°C to 34°C. (Once other *ts* mutations had been crossed out, the higher temperature was changed to 36°C).

Preliminary tests using the fluorescent antibody mithramycin donated by Dr. Belchery Pfiser Co. or the dye DAPI (Serva) suggested that both cells in a doublet contained nuclear DNA. Since cytokinesis was not occurring, it was thought that cell arrest occurred before this stage. However, *mut7* haploids were capable of forced mating *en masse* even after ten hours of high temperature incubation (Table 29). Although *mut7* strain R0428-6Ba certainly did (part b).

This observation supports the ideas that *mut7* causes cells to stop at a cell cycle stage resembling G1 (see Dutcher *et al.*; 1978, for further explanation) and that cytokinesis is unnecessary for *mut7* cells to obtain access to the mating-type step of the cell-cycle. The possibility exists that a or α factor is epistatic over the *mut7* arrest stage. However, it was noted microscopically that doublets remained doublets at 36°C during mating, so that presumptive zygotes appeared as the usual "peanut" phenotype with large cells attached at one or both ends. Finally, for *mut7/mut7* 36°C matings made following four hours pre-incubation at 36°C, substantial proportions of zygotes were observed after four hours of mating. Therefore this effect probably was not due to selection for viable diploids.

Since another cell cycle mutant, *cdc9* causes sensitivity to γ-irradiation if post-incubated at 36°C (Johnston, 1979) it was possible that *mut7* would also confer this phenotype. However, whether *mut7* strains were irradiated with four hours pre or post-incubation at 36°C, no notable γ -sensitivities were observed by spot-testing on YD plates.

Since the *mut7* terminal phenotype resembles that of several *dds* (reduced DNA synthesis (Johnson and Game, 1978) it was thought that the mutator locus might be allelic to one of the *cdc* and *dds* genes. However, *mut7* complements all established *cdc* and *dds* mutants for growth at 36 C (von Borstel, pers. comm.). The *ts* phenotype of *mut7* strains coreverts with MMS sensitivity (Table 25) and so it is possible that *mut7* is allelic to one of the *mms* mutations isolated by Prakash (1975).

TABLE 22: Ongoing cell division in a (log-phase) *MUT*$^+$ strain (XV185-6A) incubated at 36°C

Hours after innoculation	Hours after shiftup from 26°C to 36°C	Total cells counted	Proportion of budded cells	Expected colonies/ml. of culture	Observed colonies/ml. of culture	Revertants/ survivor x 10^6 -lys. medium	-his. medium
0	-2.5	272	0.72	1.6×10^6 (158)	-	- (0)	- (0)
2.5	0	339	0.65	4.1×10^6 (206)	4.9×10^6 (245)	- (0)	0.2 (1)
5.5	3	346	0.59	8.7×10^6 (217)	9.3×10^6 (232)	- (0)	0.4 (2)
9	6.5	308	0.51	20.4×10^6 (204)	20.2×10^6 (202)	0.2 (1)	0.2 (1)
13	10.5	85	0.36	3.1×10^7 (62)	3.4×10^7 (69)	- (0)	- (0)
18	15.5	57	0.26	2.3×10^7 (45)	2.3×10^7 (46)	- (0)	- (0)

* Numbers in parentheses are expected colony counts or observed colony counts, and may be compared directly with 'Total cells counted.

TABLE 23: Ongoing cell division in a (log-phase) *mut7* strain (RO1-90A) incubated at 26°C

Hours after innoculation	Total cells counted	Proportion of budded cells -	Expected colonies/ml. of culture*	Observed colonies/ml. of culture*	Revertants/ survivor x 10^6 -lys. medium	-his. medium
0	219	0.46	1.5×10^6 (150)	-	- (1)	- (1)
2.5	289	0.88	3.1×10^6 (154)	3.6×10^6 (181)	- (0)	0.8 (3)
5.5	292	0.85	6.3×10^6 (158)	5.4×10^6 (134)	1.1 (3)	1.5 (4)
9	261	0.74	15.0×10^6 (150)	15.6×10^6	1.0 (3)	0.3 (1)
13	144	0.23	29.3×10^6 (117)	20.5×10^6 (82)	0.6 (1)	1.8 (3)
18	59	0.26	23.5×10^6 (47)	24.0×10^6 (48)	- (0)	- (0)

* Numbers in parentheses are expected colony counts or observed colony counts, and may be compared directly with 'Total cells counted'

TABLE 24: Cessation of ongoing cell division in a *mut7* strain (RO1-90A) incubated at 36°C

Hours after innoculation	Hours after shiftup from 26°C to 36°C	Total cells counted	Proportion of budded cells	Expected colonies/ml. of culture*	Observed colonies/ml. of culture*	Revertants/ survivor x 10^6 -lys. medium	-his medium
0	-2.5	219	0.46	1.5×10^6 (150)	-	- (1)	- (1)
2.5	0	289	0.88	3.1×10^6 (154)	3.6×10^6 (181)	- (0)	0.8 (3)
5.5	3	232	0.83	3.8×10^6 (127)	3.3×10^6 (111)	1.4 (3)	2.2 (5)
9	6.5	174	0.95	4.5×10^6 (89)	3.5×10^6 (70)	2.9 (4)	1.4 (2)
13	10.5	169	0.95	4.3×10^6 (86)	3.0×10^6 (599)	0.8 (1)	5.0 (6)
18	15.5	154	0.95	4.0×10^6 (79)	5.3×10^4 (10.5)	48 (2)	48 (2)

* Numbers in parentheses are expected colony counts, or observed colony counts, and may be compared directly with 'Total cells counted'

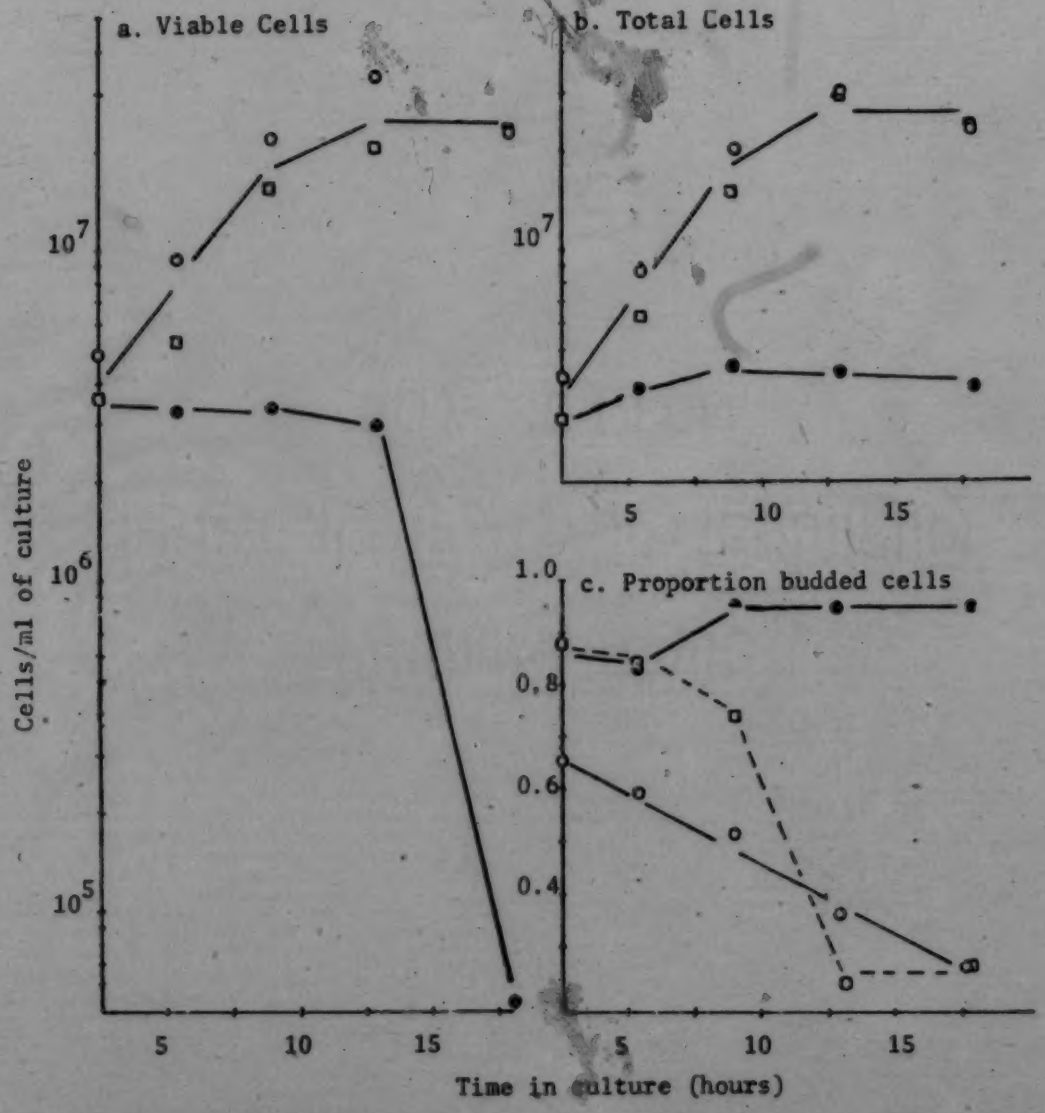


Fig. 1. Cessation of ongoing cell division, and cell death in the *mut7* strain RO1-90A incubated at 36°C (●) vs. 26°C (□). Each point represents the average value of four samples. The *mut7* cells were split into two subcultures; one of these, and a culture of *MUT*$^+$ cells (XV185-6A;○) were shifted to 36°C after 2.5 hours' incubation at 26°C.

TABLE 25: Phenotypes of revertants of *mut7-1* isolated spontaneously by selection for loss of temperature-sensitivity.

Designated *mut7* allele	Strain	Growth at 34°C	Growth at 36°C	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Prototrophs arising** on limiting lysine locus		Growth on MMS	Synergism with *mut8*	Other
7-1	RO1-90A	-	-	34 (22)	67 (0)				yes	
7-1-1	-90A^{r}1	+	-	18 (3)	13 (1)		†		no	
7-1-2	-90A^{r}2	+	-	31 (2)	42 (0)				only for *his1-7*	
7-1	RO428-6B	-	-	2 (0)	36 (0)	12	ψ	-	yes	lethal with *mut5*§
7-1-11	-6B^{r}11	+	+	0 (0)	12 (0)	2	ψ	+	no*	not lethal with mut5§
7-1	RO428-6C	-	-	68 (13)	35 (13)	6		-		
7-1-12	-6C^{r}12	+	+	8 (0)	20 (0)	1	ψ	+		
7-1 / *7-1*	RO428-6B / RO428-6C	-	-	282 (149)	35 (1)	17	ψ	-		high *his1* mitotic ¶ recombination levels
7-1-11 / *7-1-12*	RO428-6B^{r}11 / RO428-6C^{r}12	+	+	87 (37	12 (0)	1	ψ	+		normal *his1* mitotic recombination levels

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
† Average of two determinations.
ψ " " four "
§ See Results, section B3.
¶ " " " A3, esp. Table 54.
* See Appendix, Table A5.

TABLE 26: Phenotypes of spores recovered from cross RO58 : $\frac{\text{RO1-90A}}{\text{XV731-3D}}$ *

($\frac{mut7\text{-}1}{+}$ $\frac{his1\text{-}7}{his1\text{-}7}$ $\frac{+}{mut8\text{-}1}$)

Strain RO58-	Prototrophs arising** on limiting histidine		lysine		Growth at 34°C	Designated *** mut(s)
1A	116	(22)	33	(0)	+	+ *mut8*
B	43	(9)	38	(7)	-	*mut7* +
C	71	(2)	30	(1)	-	*mut7* +
D	203	(18)	18	(0)	+	+ *mut8*
2A	19	(1)	8	(0)	+	+ +
B	813	(166)	285	(2)	-	*mut7 mut8*
C	44	(12)	32	(0)	-	*mut7* +
D	174	(18)	32	(0)	+	+ *mut8*
3A	146	(49)	15	(1)	+	+ *mut8*
B	57	(2)	27	(1)	-	*mut7* +
C	21	(0)	14	(0)	+	+ +
D	939	(151)	176	(16)	-	*mut7 mut8*
4A	151	(39)	20	(0)	+	+ *mut8*
B	29	(2)	8	(0)	+	+ +
C	933	(166)	185	(7)	-	*mut7 mut8*
D	55	(5)	38	(1)	-	*mut7* +
5A	912	(254)	210	(3)	-	*mut7 mut8*
B	124	(174)	18	(0)	+	+ *mut8*
C	35	(0)	30	(0)	-	*mut7* +
D	29	(2)	26	(0)	+	+ +

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
Plates were scored after eight days.

* It should be noted that in tables indicating the phenotypes of viable spore clones recovered from sporulated diploids , "spores recovered" replaces the larger clause. Unless otherwise mentioned, each table represents a separate experiment.

*** The genotype of *mut7 mut8* is assigned to strains with very high *his1-7* (or *his1-1*, see sections A3 or B1) and *lys1-1* reversion (strains 2B 3D, 4C, and 5A). *mut8* is assigned to strains which have high histidine reversions but low lysine reversion (strains 1A, 1D, 2D, 3A, 4A, and 5B). The presence of *mut7* is associated with the segregating temperature sensitivity (see text).

TABLE 27 : Phenotypes of spores recovered from cross RO204: $\frac{RO1\text{-}90A^{r\dagger}}{XV731\text{-}3D}$

$$\left(\frac{mut7\text{-}1\text{-}1}{+} \quad \frac{+}{mut8\text{-}1} \quad \frac{his1\text{-}7}{his1\text{-}7}\right)$$

Strain RO204-	Prototrophs arising** on limiting histidine		lysine		Growth at 34°C	Designated *mut*
1A			16	(0)	all	
B			34	(0)	strains	
C			30	(0)	grew	
D			29	(0)		
2A			13	(0)		
B			16	(0)		
C			13	(0)		
3A			11	(0)		
B			22	(0)		
C			22	(0)		
4A			21	(0)		
B			17	(0)		
C			37	(0)		
D			12	(0)		
6A			19	(0)		
B			17	(0)		
C			15	(0)		
D			19	(0)		
7A	194	(179)	25	(0)		*mut8*
B	275	(130)	40	(5)		*mut8*
C	10	(4)	9	(0)		+
D	14	(4)	7	(0)		+
8A	78	(144)	32	(1)		*mut8*
B	29	(3)	10	(0)		+
C	137	(119)	33	(8)		*mut8*
D	18	(1)	9	(0)		+
9A	28	(4)	9	(0)		+
B	25	(2)	127	(116)*		+
C	146	(155)	36	(1)		*mut8*
D	181	(80)	23	(0)		*mut8*
10A	146	(120)	12	(1)		*mut8*
B	29	(3)	23	(0)		+
C	16	(2)	17	(0)		+
D	175	(208)	5	(3)		*mut8*
11A	116	(191)	18	(0)		*mut8*
B	27	(3)	11	(0)		+
C	33	(1)	17	(0)		+
D	324	(95)	16	(3)		*mut8*

TABLE 27: (continued)

Strain RO204-	Prototrophs arising** on limiting histidine		lysine		Growth at 34°C	Designated *mut*
12A	19	(1)	7	(2)	all	+
B	143	(137)	22	(0)	strains	*mut8*
C	151	(184)	15	(1)	grew	*mut8*
D	20	(0)	21	(0)		+
13A	15	(3)	10	(0)		+
B	231	(202)*	17	(0)		
C	221	(159)	26	(1)		*mut8*
D	23	(2)	15	(0)		+
14A	11	(0)	29	(0)		+
B	95	(233)	26	(0)		*mut8*
C	246	(137)	19	(1)		*mut8*
D	19	(5)	7	(1)		+
15A	279	(213)*	40	(0)		
B	31	(1)	12	(0)		+
C	25	(5)	10	(1)		+
D	192	(37)	20	(0)		*mut8*
16A	23	(3)	10	(0)		+
B	192	(158)*	26	(1)		
C	31	(6)	11	(0)		+
D	208	(137)	15	(0)		*mut8*
17A	30	(2)	8	(0)		+
B	24	(3)	8	(0)		+
C	156	(127)	16	(1)		*mut8*
D	140	(119)	28	(0)		*mut8*
18A	16	(2)	14	(0)		+
B	218	(160)	14	(0)		*mut8*
C	128	(205)	12	(0)		*mut8*
D	14	(3)	14	(0)		+
19A	30	(0)	15	(0)		+
B	25	(1)	22	(0)		+
C	187	(73)	18	(0)		*mut8*
D	78	(187)	21	(1)		*mut8*

** Plates were scored after eight days incubation. Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† Tables 26, 27 and 28 represent data obtained in one experiment.

TABLE 28: Phenotypes of spores recovered from cross RO208: $\frac{\text{RO1-90A}^r}{\text{RO1-90B}}$ ($\frac{mut7\text{-}1\text{-}2}{+}$ $\frac{+}{mut8\text{-}1}$ $\frac{hom3}{+}$ $\frac{his1\text{-}7}{+}$ $\frac{+}{his5\text{-}2}$)

Strain RO208-	Segregating alleles at *hom3*	*his1*	*his5*	Prototrophs arising** on limiting histidine		lysine		Growth at 34°C	Designated *mut(s)*	
1A	+	+	+			27	(1)	all		
B	+	+	5-2	9	(0)	9	(0)	strains		
C	-	1-7	5-2	0	(0)	15	(0)	grew		
D	-	1-7	+	642	(2)	21	(0)		*mut7 mut8* ?	
2A	+	+	5-2	3	(0)	18	(0)			
B	+	+	+			26	(0)			
C	-	1-7	+	12	(0)	5	(1)		+	+
D	-	1-7	5-2	0	(0)	25	(1)			
3A	+	+	+			54	(1)		+	+ ?
B	-	1-7	+	11	(0)	14	(0)		+	+
C	+	+	5-2	8	(0)	21	(0)			
D	-	1-7	5-2	0	(0)	25	(1)			
4A	-	1-7	+	17	(0)	11	(0)		+	+
B	+	+	+			13	(0)			
C	+	+	5-2	15	(1)	19	(0)			
D	-	1-7	5-2	0	(0)	31	(0)			
6A	-	1-7	+	164	(9)	26	(0)		+	*mut8*
B	-	1-7	5-2	0	(0)	19	(0)			
C	+	+	5-2	6	(0)	8	(0)			
D	+	+	+			14	(0)			
7A	+	1-7	5-2	0	(0)	6	(0)			
B	-	1-7	+	553	(42)	29	(1)		*mut7 mut8* ?	
C	+	+	+			17	(0)			
D	-	+	5-2	32	(0)	52	(0)		+	+ ?
8A	-	1-7	+	40	(1)	18	(0)		+	+ ?
B	+	+	5-2	11	(0)	28	(0)			
C		+	5-2	20	(0)	33	(0)			
D	-	1-7	+	6	(2)	18	(0)		+	+
9A	+	+	+			31	(0)			
B	-	1-7	5-2	0	(0)	4	(3)			
C	+	+	+			43	(0)			
D	-	1-7	5-2	0	(0)	8	(0)			
XV379-20C	+	+	5-2			136	(6)		*mut7 mut8*	
XV185-6A	-	1-7	+	27	(2)	22	(0)		+	+
YO300-2C	-	1-7	+	27	(1)	17	(0)		+	+
				21	(5)	19	(0)		+	+

** Strains were scored after eight days' incubation. Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 29: Retention of ability of *mut7* strains to force-mate following ten hours pre-incubation at 36°C

a) Complementation of *mut7,his1* haploids force-mated to *MUT⁺,his5* Haploids for four hours at 36°C--growth on -his plates at 36°C after mating.

		MUT⁺,his5-2 Strains			
		XV185-6D(a)		XV185-4A(α)	
Pre-incubated for ten hours, at--		26°C	36°C	26°C	36°C
Strain					
RO255-3A	a,*MUT⁺,his1-7*			+	+
-3C	α " "	+	+		
RO255-1B	a,*mut7,his1-7*			+	?
-4A	α " "	+	+		
RO255-3D	a,*mut7,mut8,his1-7*			+	-
-3B	α " " "	+	+		

b) Complementation of a *mut7,HOM⁺* haploid force-mated with *MUT⁺* (*mut7* reverted),*hom3* haploids for four hours--growth on -thr. plates at 36°C after mating.

Pre-incubated for ten hours, at--		26°C	26°C	36°C	36°C*
Mated for four hours, at--		26°C	36°C	26°C	36°C
Cross					
RO428-6B / RO428-6C^{r}12	a *mut7-1* / α *mut7-1-12* *HOM⁺* / *hom3-10*	+	+	+	+
RO428-6B^{r}11 / RO428-6C^{r}12	a *mut7-1-11* / α *mut7-1-12* *HOM⁺* / *hom3-10*	+	+	+	+

* Diploids from both strains mated under these conditions were sporulated successfully (>10%).

c. Macromolecular Synthesis in a *mut7* haploid at 36°C

Once it had been determined that *mut7* confers a *cdc* phenotype on cells incubated at 36°C, it was worthwhile asking whether the cessation of cell division was due to cessation of protein, RNA or DNA synthesis, or some combination of these. Uptake of radioactively labelled amino acids and bases suggested that protein and RNA synthesis continue at this temperature (Ord unpublished, von Borstel and Johnston, unpublished). Using the procedure of Johnston and Game (1978), von Borstel and Johnston determined that uptake of label into DNA in the *mut7* strain RO428-6C, and the *mut7 mut8* strain RO88-4B was curtailed, but did not abruptly cease at 36°C (unpublished). B. Cox (personal comm.) had found that another *mut7 mut8* strain had *no* residual DNA synthesis. However, it was considered that this may have been due to the presence of other *ts* loci in the strain (see section A3a). The experiments of von Borstel and Johnston weren't performed under ^{3}H-uracil pool equilibrium conditions, and so one might have expected continuous (if reduced) uptake of H^{3}-uracil if reduced DNA synthesis or DNA repair was occurring. To resolve the question of whether total DNA was increasing in *mut7* cells held at 36°C, the diphenylamine assay described by Roth (1974) was used, and adapted for haploid yeast. Since it was possible that mitochondrial DNA synthesis might also have been affecting the uptake experiments, and that this would similarly affect the diphenylamine assay, presumptive ρ^{-} strains were induced (two subcultures in 10µg/ml ethidium bromide) from the *mut7* strain RO428-6C, and the *MUT*$^{+}$ stock RO400-10C.

Large numbers of cells are required for the diphenylamine assay, and so it was necessary to grow the strains to as close to stationary phase as possible before starting the experiment. Table 30 and Figure 2 indicate that all stocks grown at 26°C attain numbers of at least 1.5×10^8 before most cells enter stationary phase. Preliminary data indicated that where cell counts were much lower than 10^7 cells/ml the diphenylamine readings would not be reliable. This is in agreement with Roth (1974).

Table 31 and Figure 3 show the effect of 36°C incubation on *mut7* strain RO428-6C (ρ^+ and ρ^-). In both strains, total DNA abruptly ceases to increase after the shift to 36°C. The *mut7* ρ^- strain was also assayed after ten hours at 36°C, to ensure that DNA synthesis had not halted merely because of temperature shock.

Table 32 and Figure 4 compare the ρ^- *mut7* strain RO428-6C with the ρ^- *MUT*$^+$ strain RO400-10C. Again the *mut7* strain ceases growing and ceases increasing in DNA content quite abruptly, but here the *MUT*$^+$ strain does so as well, at least momentarily. It is possible, then, that the rapid halt in DNA increase is dependent on some other factor. However, it is clear that, once halted, increases in DNA content do not occur in the *mut7* ρ^- strain.

Figures 5 and 6 show the changes in haploid DNA content per cell as cells approach and enter either into stationary phase, or into a 36°C *mut7* -mediated cell arrest. It is interesting to note that the decline in daltons/cell of DNA seen in all late log phase cells cultured at 26°C mimics the decrease in DNA/cell of *mut7* late log phase cells incubated at 36°C. The decreases themselves are likely to be due to

the change from two genomes per cell in rapidly dividing culture to one genome per cell as cells slow their rates of division. Note that the DNA content per cell calculated from these experiments closely resembles other estimates of DNA content per (ρ^+) yeast cell (0.8×10^{10} to 1.3×10^{10}; Hartwell, 1970), that the ρ^+ *mut7* strain appears to contain more DNA per cell than the ρ^- derivative (Figure 6), and less DNA per cell than the *MUT*$^+$ ρ^- strain (Figure 7).

TABLE 30 : Ongoing cell division in ρ^+ and ρ^- derivatives of *mut7* and *MUT*$^+$ strains incubated at 26°C

Strain	Hours after innoculation	Total cells/ml. of culture	Proportion of budded cells	Number of cells counted
RO400-10C *MUT*$^+$ ρ^+	0	4.7×10^6	0.74	188
	2	1.3×10^7	0.77	1262
	4.7	4.7 "	0.77	466
	7	9.5 "	0.60	190
	9	2.1×10^8	0.48	428
	11	3.4 "	0.36	712
	19	3.8 "	0.06	384
RO400-10C *MUT*$^+$ ρ^-	0	2.4×10^6	0.78	955
	2	4.3 "	0.76	425
	4.7	8.6 "	0.74	431
	7	1.9×10^7	0.67	379
	9	3.4 "	0.63	670
	11	6.3 "	0.58	625
	19	3.5×10^8	0.19	347
RO428-6C *mut7* ρ^+	0	2.3×10^6	0.93	922
	2	4.5 "	0.80	445
	4.7	1.4×10^7	0.91	1392
	7	3.3 "	0.88	330
	9	4.6 "	0.73	457
	11	1.3×10^8	0.77	1318
	19	1.8 "	0.95	1824
RO428-6C *mut7* ρ^-	0	2.5×10^6	0.89	986
	2	4.5 "	0.89	447
	4.7	8.4 "	0.77	422
	7	2.2×10^7	0.80	444
	9	3.7 "	0.71	739
	11	6.8 "	0.83	683
	19	4.2×10^8	0.82	419

TABLE 31 : Cessation of ongoing cell division and of net DNA increase in a culture of *mut7* cells incubated at 36°C

Strain	Culture incubated at	Hours in culture	Total cells per ml. of culture	Total cells counted	Fraction of budded cells	ΔOD/ * 10 ml. of cells	Daltons/ ml.x10^{17}	Daltons/ cell x 10^{10}
RO428-6C *mut7* ρ^+	26°C	0	8.6 x 10^6	864	0.97	0.019	1.4	1.6
		2	2.0 x 10^7	1046	0.94	0.040	3.0	1.5
		2:45	2.4 "	1207	0.95	0.040	3.0	1.3
		3:35	3.8 "	759	0.87	0.057	4.2	1.1
		6:10	7.9 "	785	0.89	0.091	6.7	0.85
		8:10	1.1 x 10^8	426	0.78	0.13	9.6	0.87
		12	2.1 "	427	0.79			
	36°C	2:45	1.9 x 10^7	972	0.88	0.034	2.5	1.3
		3:35	2.7 "	1335	0.95	0.031	2.3	0.85
		6:10	2.9 "	723	0.98	0.025	1.9	0.66
		8:10	3.4 "	853	0.95	0.035	2.6	0.77
		12	3.1 "	625	0.97			
RO428-6C *mut7* ρ^-	26°C	0	9.7 x 10^6	966	0.92	0.011	0.81	0.84
		2	1.7 x 10^7	836	0.87	0.032	2.4	1.4
		2:45	2.1 "	1072	0.76	0.027	2.0	0.95
		3:35	2.4 "	484	0.81	0.038	2.8	1.2
		6:10	5.6 "	558	0.66	0.052	3.9	0.70
		8:10	8.7 "	173	0.63	0.048	3.6	0.41
		12	2.1 x 10^8	831	0.70			
	36°C	2:45	1.8 x 10^7	892	0.74	0.027	2.0	1.1
		3:35	2.0 "	1015	0.77	0.026	1.9	0.95
		6:10	3.0 "	751	0.95	0.023	1.7	0.50
		8:10	3.4 "	839	0.97	0.023	1.7	0,57
		12	2.4 "	484	0.98	0.024	1.8	0.75

* $OD_{595nm.}$ minus $OD_{650nm.}$ for the diphenylamine reaction (average of two samples)

TABLE 32 : Cessation of ongoing cell division and of net DNA increase in a culture of *mut7*, ρ^- cells incubated at 36°C

Strain	Culture incubated at	Hours in culture	Total cells per ml. of culture	Total cells counted	Fraction of budded cells	ΔOD/* 10ml. of cells	Daltons/ ml.x 10^{17}	Daltons/ cell x 10^{10}
RO428-6C *mut7* ρ^-	26°C	0	1.6 x 10^7	1565	0.98	0.034	2.5	1.6
		2:10	3.5 "	699	0.87	0.059	4.4	1.3
		4:05	7.8 "	784	0.84	0.062	4.6	0.59
		6:45	1.3 x 10^8	1328	0.79	0.110	8.1	0.62
		10:05	1.6 "	1599	0.66	0.134	9.9	0.62
	36°C	2:35	4.7 x 10^7	944	0.82	0.048	3.6	0.77
		3:05	5.1 "	514	0.58	0.049	3.6	0.71
		3:35	6.0 "	603	0.93	0.043	3.2	0.53
		4:05	6.8 "	682	0.70	0.045	3.3	0.49
		6:45	6.9 "	692	0.92	0.031	2.3	0.33
		10:05	8.2 "	817	0.91	0.042	3.1	0.38
RO400-10C *MUT*$^+$ ρ^-	26°C	0	7.2 x 10^6	718	0.83	0.017	1.3	1.8
		2:10	1.7 x 10^7	348	0.80	0.043	3.2	1.9
	36°C	2:35	1.7 x 10^7	331	0.69	0.034	2.5	1.5
		3:05	2.5 "	245	0.66	0.029	2.1	0.84
		3:35	3.1 "	311	0.56	0.043	3.2	1.0
		4:05	3.3 "	328	0.60	0.042	3.1	0.94
		6:45	5.1 "	507	0.66	0.073	5.4	1.1
		10:05	1.2 x 10^8	1230	0.76	0.114	8.4	0.70

* OD_{595nm} minus OD_{650nm} for the diphenylamine reaction (average of two samples)

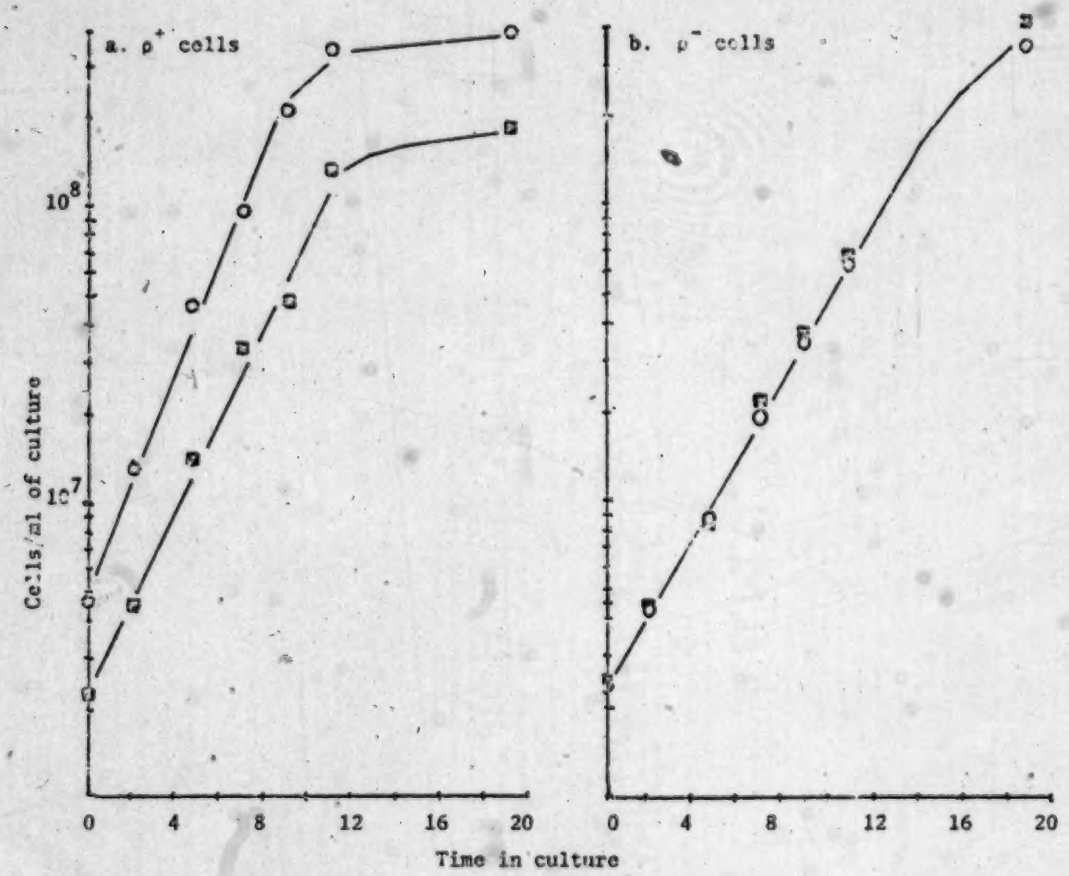


Fig. 2. Cell division in the *mut7* strain RO428-6C (■) and in the *MUT*⁺ strain RO400-10C (□) incubated at 26°C.

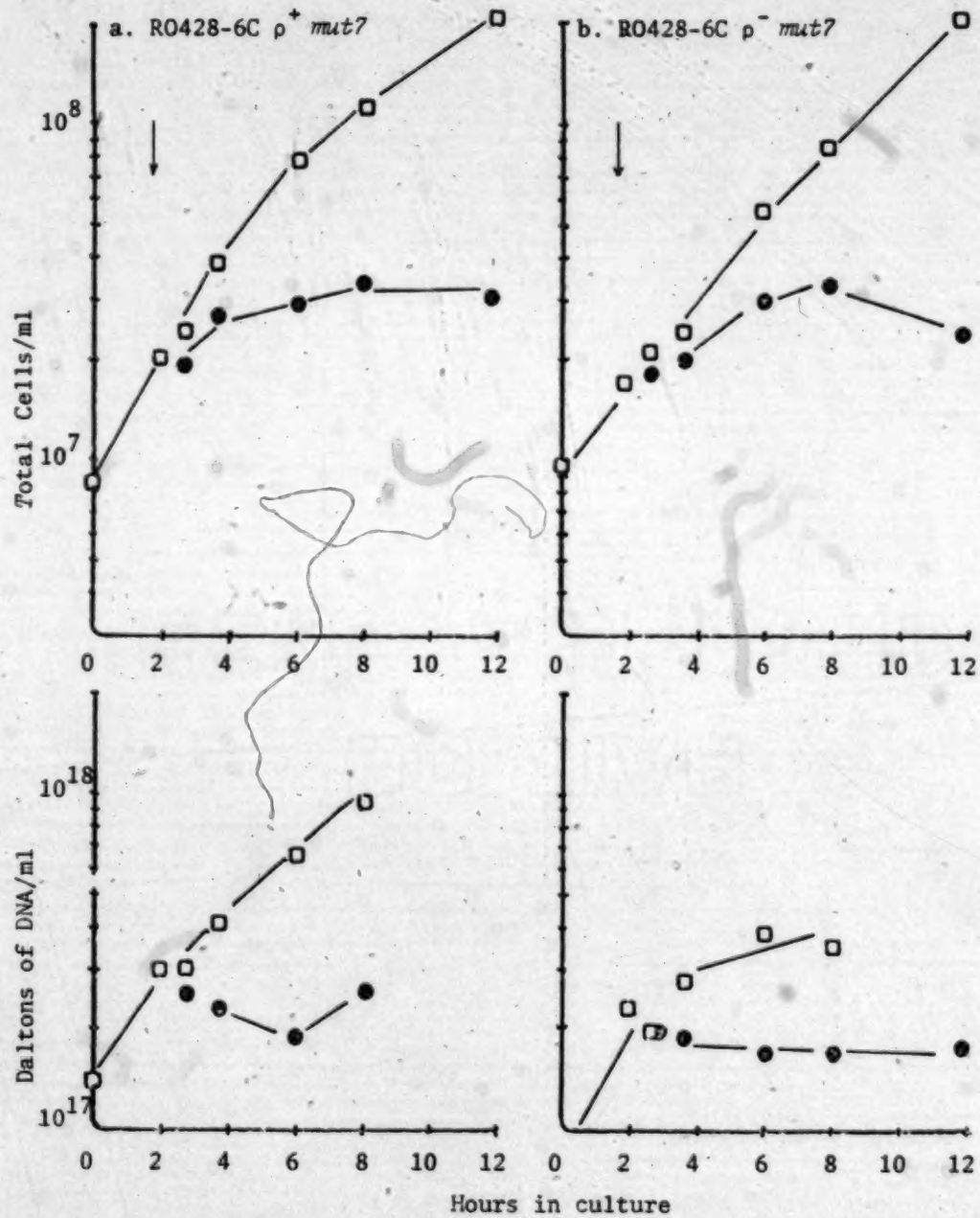


Fig. 3. Cessation of net DNA increase as measured by the diphenylamine reaction (average of two samples/point), and of cell division in *mut7*, ρ^+ cells, compared with *mut7* ρ^- cells, 36°C (●) vs. 26°C (□). The cells from each strain were split into two subcultures; one subculture from each strain was shifted to 36°C after two hours incubation at 26°C (arrows).

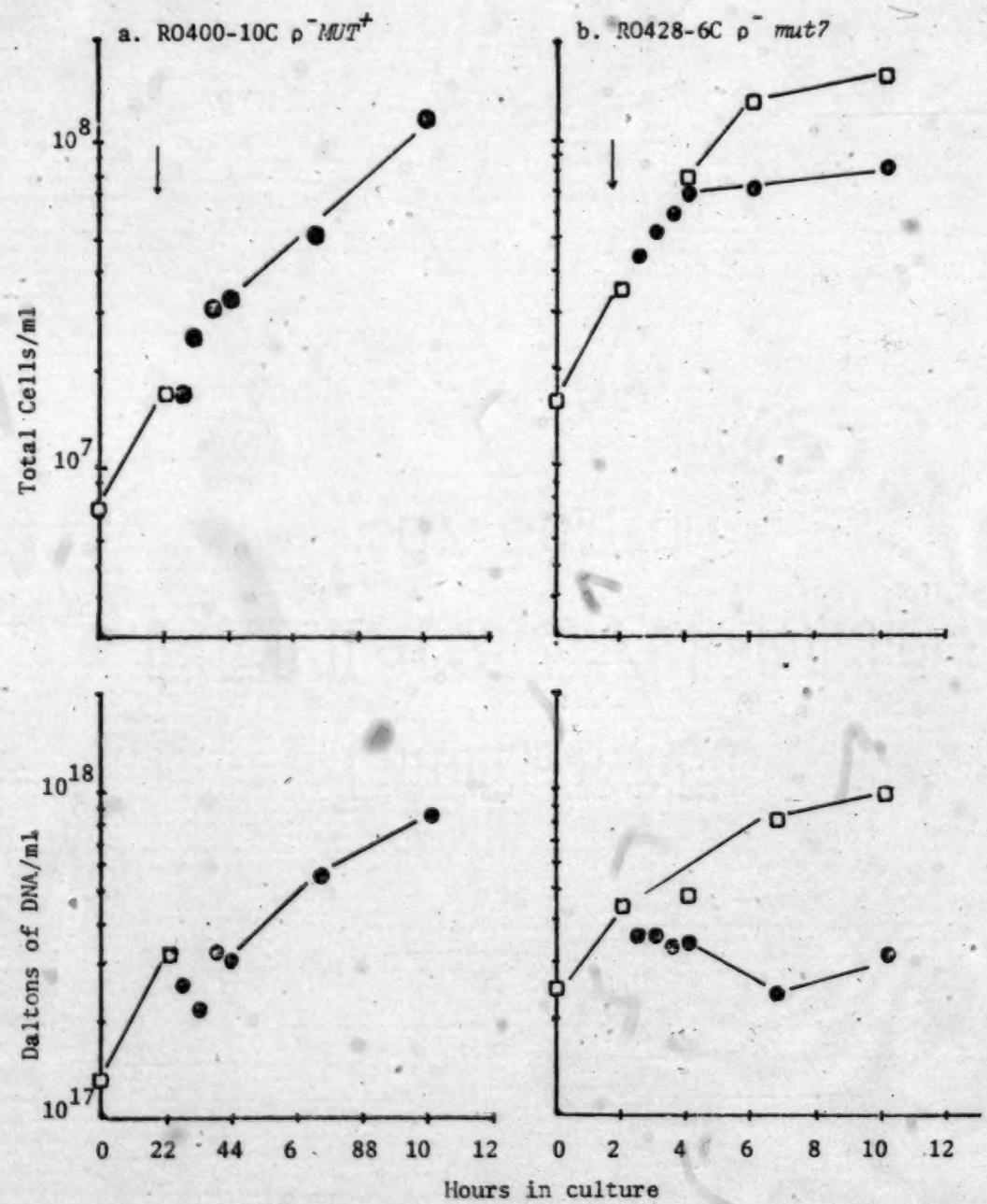


Fig. 4. Cessation of net DNA synthesis asmeasured by the diphenylamine reaction (average of two samples/point), and of cell division in *mut7*,ρ⁻ cells, compared with *MUT*⁺, ρ⁻ cells, at 36°C (●) vs. 26°C (□). The *mut7* cells were split into two subcultures; one of these, and the *MUT*⁺ culture were shifted to 36°C after two hours' incubation at 26°C (arrows).

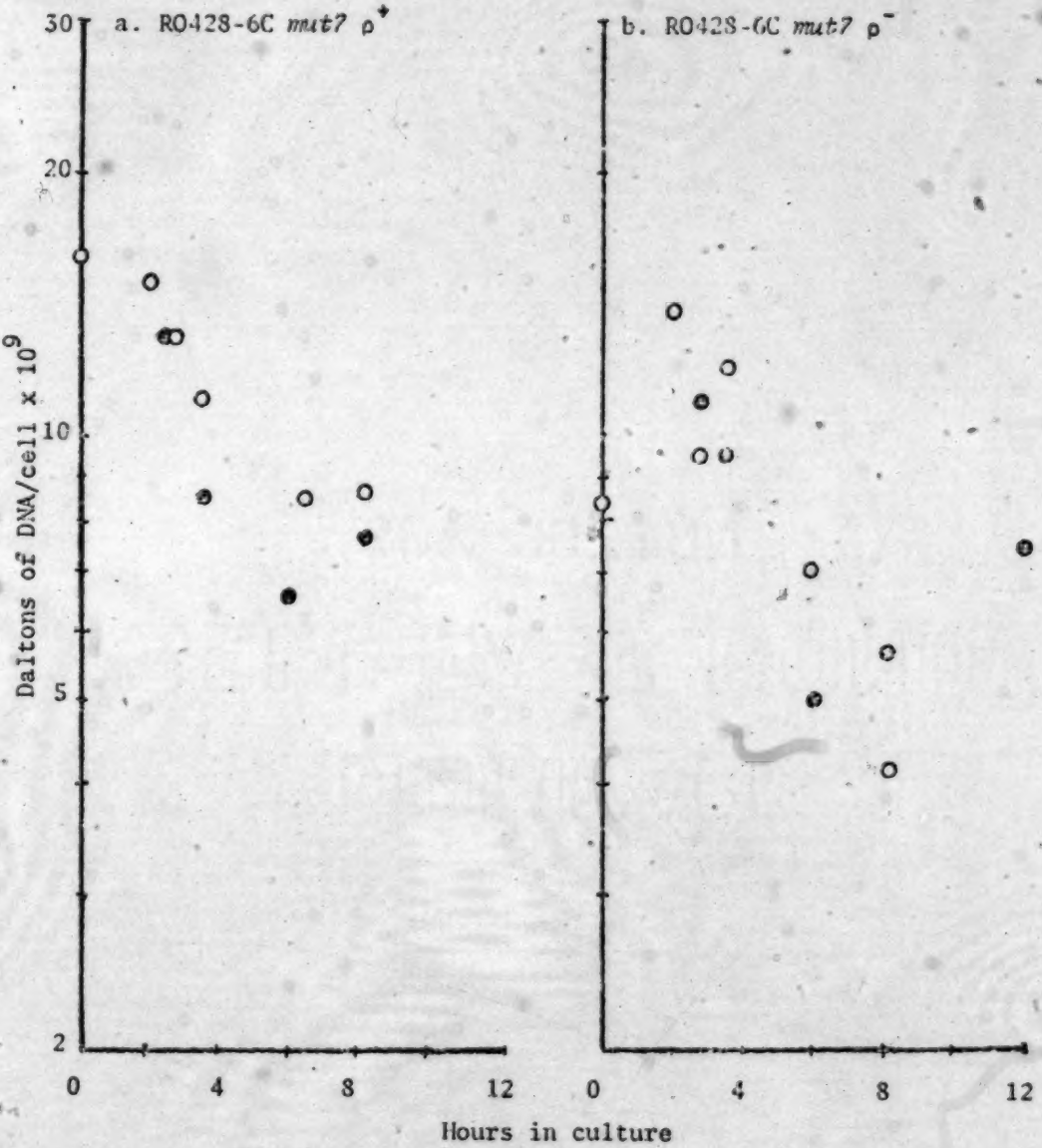


Fig. 5. Daltons of DNA per haploid cell in late log phase *mut7* ρ^+ cells (a.) or *mut7* ρ^- cells (b.) incubated at 26°C (o) or 36°C (•).

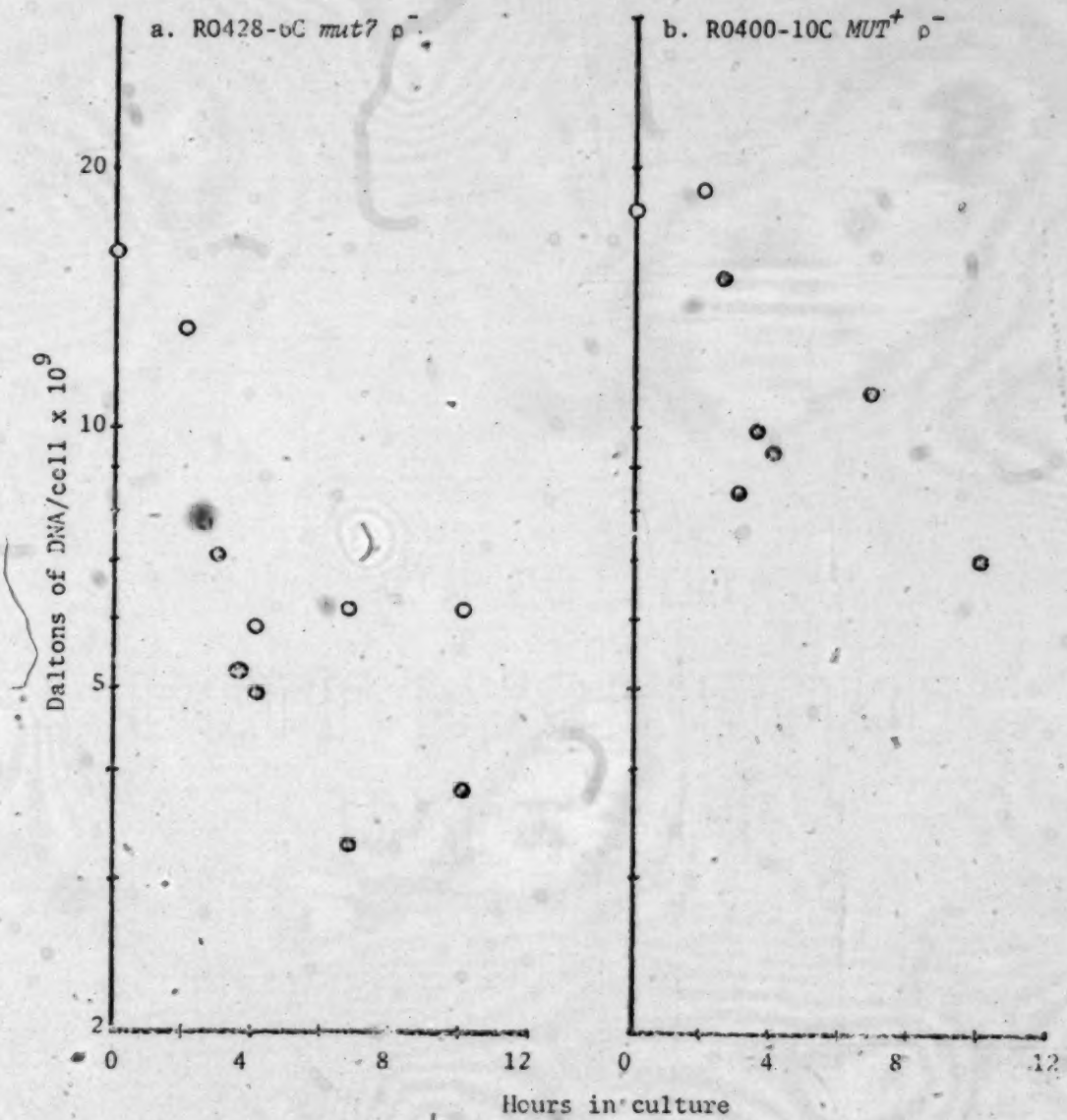


Fig. 6. Daltons of DNA per haploid cell in late log phase *mut7* ρ⁻ cells (a.) or *MUT*⁺ ρ⁻ cells incubated at 26°C (○) or at 36°C (●)

3. Spontaneous Mitotic Recombination

a. Introduction of *his1-1* into a *mut7* Strain

Although the *mut7* strain RO1-90A coreverted for temperature sensitivity and mutator phenotype, none of the revertants isolated at 34°C would grow at 36°C, and attempts to isolate revertants at 36°C were unsuccessful. It was assumed that the strains carried at least one other (36°C) temperature sensitive factor, as did all other XV stocks tested. A *MUT*$^+$ stock KC179-15A (Tables 1 and 2) which grew at 36°C was crossed to RO1-90A to attempt to separate the other temperature sensitive loci from *mut7*.

KC179-15A carried the mutant allele *his1-1*, which reverted at a low rate in the Lassie test. The allele is believed to have been caused by a frameshift mutation (Magni, 1963). As can be seen in Table 33, the diploids RO400 (KC179-15A/RO1-90A; *MUT*$^+$*/mut7*, *his1-7/his1-1*) and RO417 (KC179-15A/RO1-90A; *MUT*$^+$*/MUT*$^+$; *his1-7/his1-1*) had high Lassie scores for histidine reversion, presumably due to intragenic recombination between the two *his1* alleles. The spontaneous reversion rate for *lys1-1* in the diploid strains was approximately that of the *MUT*$^+$ haploid strains.

This enhancement of histidine prototroph formation was found to be consistent for all heteroallelic, non-mutator diploids tested (see Section A3c), with histidine Lassie scores of between 60-200 prototrophs per plate, as compared with scores of 10-20 for *his1-7/his1-7* strains and of 0-2 for *his1-1/his1-1* non-mutator diploid strains (see Tables 50 to 53). This result pointed the way toward a useful assay for measuring spontaneous recombination in mutators--another parameter of DNA repair.

Tetrad analysis of strain RO400 (Table 34) indicated that two other *ts* markers were segregating, besides *mut7*. Both had terminal phenotypes which were recognizable under the microscope. The "*tsx*" strain resembles the "*cdc11*" terminal phenotype (Hartwell, 1972), while "*tsy*" spores become single cells two or three times the diameter of cells maintained at 26°C. Cells bearing *tsx* or *tsy* were able to divide a few times before growth ceased, but the *ts* phenotype was clearly discernible on replica plates incubated for two days at 36°C. These strains could still grow at 34°C, while *mut7*-bearing strains could not.

To eliminate *tsx* and *tsy* from the *mut7* background, the following protocols were employed. The first, summarized in Table 35 (and Tables 36-48), determined whether any strain carried another *ts* locus. Then the *mut7* locus was outcrossed until no other *ts* markers segregated.

b. Properties of the *his1-1* allele

The process of eliminating the *ts* mutations genetically permitted us to analyse reversion of the *his1-1* allele extensively (Tables 36 to 48). Table 36 shows the segregation of *mut7* and *his1-7*. Tables 37 and 44 show the lack of effect of *mut7* on *his1-1* strains (a mean of 1.3 reversions per plate compared with 0.9 reversions per plate for MUT^+ strains can be calculated from the data in Table 37). In Tables 38,39 and 45, the *his1-1* allele can be seen to segregate 2:2, as a low histidine Lassie score, from the *his1-7* or the HIS^+ alleles in homozygous MUT^+ strains.

The *his1-1* allele was then analysed in a *mut8* and *mut7 mut8* background. Table 40 shows the usual effect of *mut7* and/or *mut8* on

his1-7 revertability. However, in Table 41, note that the *his1-1* Lassie scores in RO406-3A and 4B (which presumably are *mut7 mut8*) appear to show greater than 20-fold enhancement over what would be expected in *MUT*$^+$ or *mut7* homozygotes. It would seem that the double mutator mutant acts synergistically on *his1-1* (see also Table 58).

Heterozygotes for *mut8* segregate for high *his1-7* mutation (Table 42). In crosses heteroallelic for *his1* and heterozygous for *mut8* alone (Table 43), the higher *his1-1* reversion noted in Table 41 is seen again. Crosses 107 and 108 (control crosses for mutator allelisms, Tables 59 and 60) confirm that *mut8* does cause a five- to six-fold enhancement of *his1-1* Lassie reversion. Homozygosity for *mut7* has no effect on the segregation of the *his1-1* phenotype (Table 46).

Other loci segregating in these crosses have been included in several tables. *cry1*, in the homozygous condition, has been found to enhance spontaneous reversion of *lys1-1* (Morrison, 1978). The presence of this marker appeared to have no effect on any haploid strains tested here (Tables 38, 39, and 47). Similarly, *ade2*, a marker affecting DNA metabolism doesn't seem to influence reversion scores (Tables 38 and 47).

The segregation of *his1-7*, *his1-1* or *HIS1*$^+$ alleles does not appear to affect reversion of *lys1-1* in *mut7*, *mut8* or *MUT*$^+$ strains. [The other auxotrophic loci also have little effect on mutation. When stocks containing only *his1-7* were constructed for auxotroph mutation studies (see section A1c), the absence of other markers appeared to have no effect on *his1-7* reversion (data not shown).]

Tables 47 and 48 describe the phenotypes of tetrads segregating only for *mut7*, *hom3*, *his1*, and *arg4*. These tables may be useful for reference when comparing crosses of *mut7* to other mutators.

TABLE 33: Phenotypes of diploid strains constructed to study temperature sensitivities observed in *mut7* haploids, and heteroallelism at *his1* (experiment #1)

Strain number	Haploid Strain	Diploid strain	Pertinant genotype	Growth at 36°C	Growth at 34°C	Prototrophs arising* on limiting histidine		Prototrophs arising* on limiting lysine	
RO1	90A		*mut7 his1-7*	-	-	34	(22)	65	(0)
XV185	6A		*MUT+ his1-7*	-	+	14	(1)	15	(1)
KF179	15A		*MUT+ his1-1*	+	+	0	(1)	27	(0)
RO400		RO1-90A / KF179-15A	*mut7 / + his1-7 + / + his1-1*	+	+	98	(14)	19	(0)
						93	(43)	15	(3)
						89	(10)	17	(0)
RO417		XV185-6A / KF179-15A	*MUT+ / MUT+ his1-7 + / + his1-1*	+	+	93	(7)	20	(0)
						102	(22)	20	(2)
						69	(5)	5	(2)

* Numbers of prototrophs arising prior to growth on limiting medium are parenthesized.

TABLE 34: Phenotypes of spores recovered from cross RO400 ($\frac{mut7}{+}$ $\frac{hom3\text{-}10\ his1\text{-}7}{+\quad +}$ $\frac{+}{+\ his1\text{-}1}$)

Strain RO400-	Segregating allele at *cry1**	Segregating allele at *hom3*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Survival at 36°C	Survival at 34°C	Designated mutator
2A	+	+	0	(0)	54	(1)	-	-	*mut7*
B	+	+	2	(0)	64	(2)	-	-	*mut7*
C	-	-	37	(3)	8	(0)	+	+	+
D	-	-	55	(0)	13	(1)	+	+	+
3A	+	-	56	(0)	15	(0)	-	+	+
B	-	+	1	(0)	12	(2)	+	+	+
C	-	-	69	(0)	29	(0)	-	-	*mut7*
D	+	+	0	(0)	60	(0)	-	-	*mut7*
$4A^{\rho}$	+	-	18	(1)	14	(2)	+/-	+	+
B^{ρ}	+	-	30	(1)	8	(0)	-	+/-	+
C^{ρ}	-	+	7	(0)	29	(1)	-	-	*mut7*
D^{ρ}	-	+	1	(1)	14	(0)	-	-	*mut7*
5A	+	+	2	(0)	12	(0)	-	+	+
B	+	+	3	(0)	61	(0)	-	-	*mut7*
C	-	-	11	(0)	11	(0)	+	+	+
D	-	-	50	(4)	33	(0)	-	-	*mut7*
6A	+	-	66	(4)	40	(1)	-	-	*mut7*
B	+	+	1	(0)	45	(0)	-	-	*mut7*
C	-	+	0	(0)	15	(0)	+/-	+	+
D	-	-	21	(1)	12	(0)	-	+	+
7A	-	-	135	(3)	53	(3)	-	-	*mut7*
B	-	-	24	(0)	12	(1)	+	+	+
C	+	+	0	(0)	46	(48)	-	-	*mut7*
D	+	+	1	(0)	18	(0)	+	+	+
8A	+	-	106	(5)	69	(0)	-	-	*mut7*
B	-	+	1	(0)	54	(0)	-	-	*mut7*
C	-	-	16	(0)	11	(0)	+/-	+	+
D	+	+	1	(0)	7	(0)	+/-	+	+
9A	+	+	0	(0)	35	(0)	-	-	*mut7*
B	-	+	1	(0)	69	(0)	-	-	*mut7*
C	+	-	38	(1)	10	(0)	+	+	+
D	-	-	32	(0)	22	(0)	-	+	+

TABLE 34: (continued)

Strain RO400-	Segregating alleles at *cry1**	*hom3*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator
10A	+	-	105	(8)	50	(3)	-	-	*mut?*
B(*his*$^+$)	+	+			10	(1)	+	+	+
C	-	-	13	(1)	10	(0)	+	+	+
D	-	+	2	(0)	70	(1)	-	-	*mut?*
11A	-	+	0	(0)	14	(0)	-	+	+
B	+	-	82	(1)	43	(1)	-	-	*mut?*
C	+	-	46	(1)	13	(0)	+	+	+
D	-	+	2	(0)	69	(1)	-	-	*mut?*
12A†	-	-	3	(0)	10	(1)	+	+	+
B	-	-	97	(3)	38	(2)	-	-	*mut?*
C†	+	+	38	(5)	14	(0)	+/-	+	+
D	+	+	1	(0)	51	(1)	-	-	*mut?*
13A	+	+	1	(0)	14	(0)	+	+	+
B	-	-	88	(4)	43	(4)	-	-	*mut?*
C	-	+	2	(0)	105	(1)	-	-	*mut?*
D	+	-	25	(0)	7	(0)	+	+	+
14A$^\rho$	+	-	75	(5)	23	(0)	-	-	*mut?*
B	+	+	0	(0)	15	(0)	-	+	+
C	-	+	0	(0)	52	(0)	-	-	*mut?*
D	-	-	16	(0)	10	(0)	-	+	+
15A	+	+	1	(0)	6	(0)	-	+	+
B	-	+	4	(0)	71	(0)	-	-	*mut?*
C	-	-	64	(1)	45	(0)	-	-	*mut?*
D	+	-	53	(1)	11	(0)	+/-	+	+
16A	+	-	27	(17)	15	(0)	+	+	+
B	+	+	0	(0)	14	(0)	+	+	+
C	-	-	73	(5)	35	(0)	-	-	*mut?*
D	-	+	2	(0)	44	(4)	-	-	*mut?*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

$^\rho$ These strains failed to grow when plated on YG medium

† Recombination has probably occured between *hom3-10* and *his1*.

* Note the failure of segregating cryptopleurine resistance to enhance spontaneous reversion in *MUT*$^+$ or *mut?* haploids.

TABLE 35: Temperature-sensitivities in tetrad RO400-8 -- a complementation Matrix and its interpretation

A. Strains crossed and diploids created.

	RO400-8A *mut7*	RO400-8D *mut*$^+$	XV731-3D *mut8*
RO400-8B *mut7*	RO411	RO409	RO406
RO400-8C *mut*$^+$	RO412	RO410	RO407
XV731-10A *mut8*	RO405	RO408	

B. Complementation at 36°C.

	RO400-8A	RO400-8D	XV731-3D
RO400-8B	-*	-	-
RO400-8C	+/-	+	+
XV731-10A	+	-	-

C. Interpretation: let *tsx* and *tsy* be two temperature-sensitivities which curtail growth at 36°C, but not at 34°C.

	RO400-8A *mut7 tsy*	RO400-8D *tsx*	XV731-3D *tsx*
RO400-8B *mut7 tsx*	$\frac{mut7}{mut7}\ \frac{tsy}{+}\ \frac{+}{tsx}$	$\frac{+}{mut7}\ \frac{tsx}{tsx}$	$\frac{+}{mut7}\ \frac{tsx}{tsx}$
RO400-8C *tsy*	$\frac{mut7}{+}\ \frac{tsy}{tsy}$	$\frac{tsx}{+}\ \frac{+}{tsy}$	$\frac{tsx}{+}\ \frac{+}{tsy}$
XV731-10A *tsx*	$\frac{mut7}{+}\ \frac{tsy}{+}\ \frac{+}{tsx}$	$\frac{tsx}{tsx}$	$\frac{tsx}{tsx}$

* Strain RO411 (*mut7*/*mut7*) also failed to grow at 34°C.

TABLE 36: Phenotypes of spores recovered from cross RO401 ($\frac{mut7\ hom3\text{-}10\ his1\text{-}7}{+\quad +\quad +}$)

Strain RO401-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Survival at 36°C	Survival at 34°C	Designated mutator
1A	-	-	89	(2)	41	(1)	-	-	*mut7*
B	+	+			47	(0)	-	-	*mut7*
C	+	+			18	(0)	+	+	+
D	-	-	62	(7)	16	(2)	+	+	+
2A†	-	+			9	(0)	+	+	+
B†	+	-	12	(0)	33	(0)	-	-	*mut7*
C	-	-	29	(1)	6	(0)	-	+	+
D	+	+			31	(0)	-	-	*mut7*
3A	-	-	84	(0)	46	(0)	-	-	*mut7*
B	+	+			21	(2)	+	+	+
C	-	-	47	(1)	9	(0)	-	+	+
D	+	+			47	(0)	-	-	*mut7*
4A	-	-	33	(0)	11	(0)	-	+	+
B	+	+			13	(0)	+	+	+
C	+	+			55	(2)	-	-	*mut7*
D	-	-	135	(5)	45	(1)	-	-	*mut7*
5A	-	-	24	(0)	17	(0)	+	+	+
B	+	+			21	(1)	+	+	+
C	-	-	94	(2)	59	(0)	-	-	*mut7*
D	+	+			56	(0)	-	-	*mut7*
6A	+	+			44	(0)	-	-	*mut7*
B	+	+			21	(0)	-	+	+
C	-	-	29	(1)	19	(0)	+	+	+
D	-	-	105	(2)	58	(1)	-	-	*mut7*
7A	+	+			11	(0)	+	+	+
B	-	-	54	(0)	31	(0)	-	-	*mut7*
C	-	-	81	(0)	52	(0)	-	-	*mut7*
D	+	+			14	(0)	-	+	+
8A	+	+			13	(0)	+	+	+
B	+	+			61	(0)	-	-	*mut7*
C	-	-	31	(0)	17	(1)	+	+	+
D	-	-	69	(0)	42	(0)	-	-	*mut7*
9A	-	-	37	(2)	13	(0)	+	+	+
B	-	-	84	(8)	42	(0)	-	-	*mut7*
C	+	+			31	(0)	+	+	+
D	+	+			64	(1)	-	-	*mut7*

TABLE 36: (continued)

Strain RO401-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator
10A	+	+			80	(1)	-	-	*mut?*
B	+	+			23	(0)	+	+	+
C	-	-	102	(3)	33	(1)	-	-	*mut?*
D	-	-	27	(0)	8	(0)	-	+	+
11A	-	-	99	(1)	69	(0)	-	-	*mut?*
B	+	+			48	(0)	-	-	*mut?*
C	+	+			15	(0)	-	+	+
D	-	-	30	(0)	10	(1)	+	+	+
12A	-	-	39	(1)	12	(0)	+	+	+
B	+	+			52	(1)	-	-	*mut?*
C	+	+			76	(2)	-	-	*mut?*
D	-	-	15	(5)	9	(1)	+	+	+
13A	+	+			35	(0)	-	-	*mut?*
B	+	+			11	(0)	-	+	+
C$^{\psi}$	-	-	21	(0)	14	(0)	-	-	*mut?*
D	-	-	28	(0)	23	(1)	+	+	+
14A	+	+			21	(0)	-	+	+
B	-	-	108	(3)	45	(0)	-	-	*mut?*
C†	-	+			14	(0)	-	+	+
D†	+	-	93	(3)	55	(1)	-	-	*mut?*
15A	-	-	39	(0)	7	(0)	-	+	+
B	+	+			73	(0)	-	-	*mut?*
C	-	-	132	(3)	54	(1)	-	-	*mut?*
D	+	+			17	(0)	-	+	+
16A	+	+			56	(0)	-	-	*mut?*
B	+	+			55	(2)	-	-	*mut?*
C	-	-	19	(3)	14	(0)	-	+	+
D	-	-	46	(0)	6	(0)	+	+	+
17A	+	+			21	(0)	+	+	+
B	-	-	47	(0)	44	(0)	-	-	*mut?*
C	+	+			22	(0)	-	+	+
D	-	-	109	(0)	60	(1)	-	-	*mut?*
18A	+	+			52	(0)	-	-	*mut?*
B$^{\psi}$	-	-	57	(0)	30	(0)	-	-	*mut?*
C	-	-	15	(0)	12	(0)	+	+	+
D	+	+			27	(0)	-	+	+

TABLE 36: (continued)

Strain RO401-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator
19A	+	+			29	(0)	+	+	+
B	-	-	149	(138)	65	(2)	-	-	*mut7*
C	-	-	34	(0)	13	(0)	+	+	+
D	+	+			56	(1)	-	-	*mut7*
20A$^{\psi}$	-	-	67	(4)	25	(0)	-	-	*mut7*
B	-	-	25	(0)	11	(0)	+	+	+
C	+	+			19	(0)	+	+	+
D	+	+			39	(0)	-	-	*mut7*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† *hom3-his1* recombinants

ψ slow-growing strains

TABLE 37: Phenotypes of spores recovered from cross RO402 ($\frac{mut?}{+}$ $\frac{HOM^+\ his1\text{-}1}{HOM^+\ his1\text{-}1}$)

Strain RO402-	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	Survival at 34°C	Designated mutator
1A	1	(0)	9	(0)	+	+	+
B	0	(0)	41	(0)	-	-	*mut?*
C	0	(0)	10	(0)	+	+	+
D	0	(0)	56	(0)	-	-	*mut?*
2A	1	(0)	28	(1)	-	-	*mut?*
B	0	(0)	10	(1)	+	+	+
C	0	(0)	15	(0)	-	+	+
D	3	(0)	67	(1)	-	-	*mut?*
3A	0	(0)	57	(1)	-	-	*mut?*
B	1	(0)	12	(1)	+/-	+	+
C	0	(0)	4	(0)	+	+	+
D	5	(0)	60	(0)	-	-	*mut?*
4A	1	(0)	60	(6)	-	-	*mut?*
B	0	(0)	39	(4)	-	-	*mut?*
C	2	(0)	11	(0)	+	+	+
D	0	(0)	16	(0)	+	+	+
5A	2	(0)	56	(0)	-	-	*mut?*
B	1	(0)	19	(7)	+	+	+
C	2	(0)	68	(0)	-	-	*mut?*
D	1	(0)	12	(0)	+	+	+
6A	0	(0)	20	(0)	+	+	+
B	2	(0)	19	(0)	+	+	+
C	4	(0)	41	(0)	-	-	*mut?*
D	2	(0)	48	(0)	-	-	*mut?*
7A	0	(0)	40	(0)	-	-	*mut?*
B	1	(0)	39	(1)	-	-	*mut?*
C	0	(0)	14	(0)	+	+	+
D	0	(0)	17	(0)	+	+	+
8A^p	2	(0)	6	(0)	+	+	+
B^p	0	(0)	40	(0)	-	-	*mut?*
C	0	(0)	11	(0)	+	+	+
D	1	(0)	23	(0)	-	-	*mut?*
9A	0	(0)	38	(8)	-	-	*mut?*
B	0	(0)	15	(0)	+	+	+
C	0	(0)	46	(7)	-	-	*mut?*
D	1	(0)	20	(0)	+	+	+
10A^p	1	(0)	25	(0)	-	-	*mut?*
B	1	(0)	14	(0)	+	+	+
C	1	(0)	44	(0)	-	-	*mut?*
D	1	(0)	8	(0)	-	+	+

TABLE 37: (continued)

Strain RO402-	Prototrophs arising** on limiting histidine		lysine		Survival a 36°C	34°C	Designated mutator
11A	0	(0)	14	(0)	+	+	+
B	0	(0)	55	(0)	-	-	*mut?*
C	2	(0)	39	(0)	-	-	*mut?*
D	1	(0)	8	(0)	-	+	+
12A	2	(0)	58	(0)	-	-	*mut?*
B	0	(0)	39	(0)	-	-	*mut?*
C	2	(0)	21	(0)	+	+	+
D	0	(0)	11	(0)	-	+	+
13A	4	(0)	70	(2)	-	-	*mut?*
B	3	(0)	58	(0)	-	-	*mut?*
C	0	(0)	11	(0)	-	+	+
D	0	(0)	4	(0)	+	+	+
14A	2	(0)	55	(0)	-	-	*mut?*
B	1	(0)	5	(0)	+	+	+
C	2	(0)	68	(0)	-	-	*mut?*
D	0	(0)	14	(0)	+	+	+
15A	1	(2)*	22	(1)	-	-	*mut?*
B	0	(0)	23	(0)	+	+	+
C$^{\rho}$	12	(0)	9	(0)	+	+	+
D	1	(0)	31	(2)	-	-	*mut?*
16A	2	(0)	45	(1)	-	-	*mut?*
B	0	(0)	9	(0)	+	+	+
C	3	(0)	26	(0)	-	-	*mut?*
D	0	(0)	7	(0)	-	+	+
17A	0	(0)	28	(0)	-	-	*mut?*
B	0	(0)	11	(0)	+	+	+
C	1	(0)	39	(0)	-	-	*mut?*
D	2	(0)	17	(0)	+/-	+	+
18A	3	(0)	25	(0)	+	+	+
B	0	(0)	18	(0)	+	+	+
C	1	(0)	28	(0)	-	-	*mut?*
D	1	(0)	29	(0)	-	-	*mut?*
19A	1	(0)	13	(0)	+	+	+
B	1	(0)	56	(1)	-	-	*mut?*
C	0	(0)	12	(0)	-	-	*mut?*
D	0	(0)	14	(1)	+	+	+
20A	1	(1)*	15	(0)	+	+	+
B	2	(0)	11	(0)	+	+	+
C	1	(0)	53	(0)	-	-	*mut?*
D	0	(0)	15	(1)	-	-	*mut?*

** Pre-existing prototrophs are parenthesized.
* 'Jackpot' ρ Strains unable to grow on YG medium.

TABLE 38: Phenotypes of spores recovered from cross RO403

$$\left(\frac{MUT^{+}}{MUT^{+}}\ \frac{hom3\text{-}10\ his1\text{-}7\ +}{+\ +\ his1\text{-}1}\right)$$

Strain RO403-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Survival at 36°C	Survival at 34°C	Segregating alleles at *cry1*	Segregating alleles at *ade2*
1A	+	1-1	1	(0)	11	(3)	+	+	-	+
B	-	1-7	18	(6)	14	(0)	-	+	+	-
C	-	1-7	24	(19)	15	(0)	-	+	-	-
D	+	1-1	2	(0)	19	(1)	+	+	+	+
2A	+	1-1	2	(0)	15	(0)	+	+	+	+
B	-	1-7	26	(0)	35	(27)*	-	+	-	-
C	-	1-7	31	(0)	10	(0)	+	+	+	+
D	+	1-1	1	(0)	17	(25)	-	+	-	-
3A	-	1-7	13	(0)	6	(0)	-	+	-	+
B	+	1-1	0	(0)	14	(1)	+	+	+	-
C	+	1-1	0	(0)	20	(0)	-	+	+	-
D	-	1-7	6	(0)	11	(1)	+	+	-	+
4A	+	1-1	1	(0)	9	(0)	+	+	-	-
B†	-	1-7	21	(0)	7	(0)	+/-	+	+	+
C†	-	1-1	0	(0)	15	(1)	+/-	+	+	-
D†	+	1-7	14	(0)	11	(1)	+	+	-	+
5A	+	1-1	0	(0)	17	(0)	+	+	+	+
B	-	1-7	34	(0)	12	(0)	-	+	+	-
C	+	1-1	1	(0)	14	(0)	-	+	-	-
D	-	1-7	9	(0)	4	(2)	+	+	-	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† *hom3-his1* recombinants

TABLE 39: Phenotypes of spores recovered from cross RO404

$$\left(\frac{MUT^+}{MUT^+}\ \frac{HOM3^+}{HOM3^+}\ \frac{his1\text{-}1}{+}\right)$$

Strain RO404-	Segregating alleles at *cry1*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Survival at 36°C	Survival at 34°C
1A	-	-	2	(0)	13	(0)	-	+
B	+	+			27	(5)	+	+
C	+	+			10	(1)	+	+
D	-	-	1	(0)	17	(1)	-	+
2A	+	-	2	(0)	15	(0)	+	+
B	+	+			23	(1)	+	+
C	-	+			22	(0)	-	+
D	-	-	3	(0)	14	(0)	-	+
3A	+	+			17	(0)	+/-	+
B	-	+			10	(2)	+	+
C	+	-	2	(2)*	19	(0)	-	+
D	-	-	0	(0)	11	(0)	+	+
4A	-	+			15	(0)	+	+
B	+	-	1	(0)	96	(1)	-	+
C	-	-	0	(0)	14	(0)	+	+
D	+	+			9	(0)	+	+
5A	+	+			6	(0)	-	+
B	+	+			21	(0)	-	+
C	-	-	2	(0)	14	(0)	+	+
D	-	-	1	(0)	11	(0)	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot'(In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 40: Phenotypes of spores recovered from cross RO405 (*mut7* / + + / *mut8* *hom3-10 his1-7* / *hom3-10 his1-7*)

Strain RO405-	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival at 34°C	Designated mutator(s)	
1A	19 (0)	8 (0)	+	+	+	+
B	841 (260)	75 (4)	-	-	*mut7*	*mut8*
C	947 (117)	96 (11)	-	-	*mut7*	*mut8*
D	32 (0)	7 (0)	+	+	+	+
2A	254 (18)	24 (0)	-	+	+	*mut8*
B	67 (0)	38 (2)	-	-	*mut7*	+
C	870 (107)	103 (2)	-	-	*mut7*	*mut8*
D	435 (445)*	10 (0)	+	+	+	+
3A	24 (0)	8 (0)	+	+	+	+
B	139 (13)	18 (0)	-	+	+	*mut8*
C	78 (7)	32 (0)	-	-	*mut7*	+
4A	24 (18)	10 (0)	+	+	+	+
B	87 (13)	22 (0)	-	+	+	*mut8*
C	989 (220)	98 (14)	-	-	*mut7*	*mut8*
5A	31 (0)	14 (0)	-	+	+	+
B	340 (15)	94 (5)	-	-	*mut7*	*mut8*
C	155 (10)	12 (0)	+	+	+	*mut8*
D	30 (5)	41 (0)	-	-	*mut7*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 41: Phenotypes of spores recovered from cross RO406

$$\left(\frac{mut7}{+}\ \frac{+}{mut8}\ \frac{+}{hom3\text{-}10}\ \frac{+}{his1\text{-}7}\ \frac{his1\text{-}1}{+}\right)$$

Strain RO406-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator(s)†	
1A	-	1-7	541	(423)*	5	(0)	-	+	+	
B	+	1-1	8	(0)	18	(0)	-	+	+	
C	-	1-7	33	(0)	10	(0)	-	-	*mut7*	+
D	+	1-1	0	(0)	25	(0)	-	-	*mut7*	
2A	+	1-1	0	(0)	17	(0)	-	+	+	
B	-	1-7	23	(0)	13	(0)	-	-	*mut7*	+
C	-	1-7	1410	(108)	71	(2)	-	-	*mut7*	*mut8*
D	+	1-1	8	(0)	25	(0)	-	+	+	
3A	+	1-1	76	(2)	102	(4)	-	-	*mut7*	*mut8*
B	+	1-1	2	(0)	11	(0)	-	+	+	
C	-	1-7	142	(6)	24	(0)	+	+	+	*mut8*
D	-	1-7	18	(1)	12	(0)	-	-	*mut7*	+
4A	-	1-7	126	(13)	7	(0)	-	+	+	*mut8*
B	+	1-1	67	(0)	105	(37)	-	-	*mut7*	*mut8*
C	+	1-1	0	(0)	6	(0)	+	+	+	
D	-	1-7	50	(4)	43	(1)	-	-	*mut7*	+
5A	+	1-1	6	(0)	21	(0)	+	+	+	
B	+	1-1	4	(0)	3	(2)	-	+	+	
C	-	1-7	64	(2)	28	(1)	-	-	*mut7*	+
D	-	1-7	15	(0)	6	(0)	-	-	*mut7*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (Inthese cases unparenthesized numbers include pre-existing prototrophs.)

† *mut7 mut8* strains are determined by *lys1-1* reversion and temperature sensitivity.

TABLE 42: Phenotypes of spores recovered from cross RO407

$$\left(\frac{+}{mut8}\ \frac{hom3\text{-}10\ his1\text{-}7}{hom3\text{-}10\ his1\text{-}7}\right)$$

Strain RO407-	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	Survival at 34°C	Designated mutator
1A	49	(3)	10	(0)	+	+	+
B	28	(0)	12	(15)	-	+	+
C	214	(16)	11	(0)	+	+	*mut8*
D	269	(12)	13	(0)	-	+	*mut8*
2A	279	(68)	12	(0)	-	+	*mut8*
B	226	(1)	14	(0)	+	+	*mut8*
C	14	(0)	7	(0)	-	+	+
D	24	(0)	6	(0)	+	+	+
3A	178	(12)	18	(0)	+	+	*mut8*
B	19	(0)	13	(0)	-	+	+
C	17	(0)	18	(0)	+	+	+
D	443	(67)	118	(98)*	-	+	*mut8*
4A	205	(25)	18	(0)	+	+	*mut8*
B	22	(0)	15	(0)	-	+	+
C	13	(0)	11	(0)	-	+	+
D	337	(17)	19	(1)	+	+	*mut8*
5A	397	(36)	17	(1)	-	+	*mut8*
B	7	(1)	15	(0)	+	+	+
C	266	(30)	14	(0)	-	+	*mut8*
D	26	(0)	8	(0)	+	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 43: Phenotypes of spores recovered from cross RO408

$$\left(\frac{+}{mut8}\ \frac{+}{hom3\text{-}10}\ \frac{+}{his1\text{-}7}\ \frac{his1\text{-}1}{+}\right)$$

Strain RO408-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator
1A	-	1-7	28	(0)	17	(0)	-	+	+
B	+	1-1	1	(0)	6	(0)	-	+	
C	+	1-1	17	(0)	16	(0)	-	+	
D	-	1-7	178	(8)	15	(0)	+	+	*mut8*
2A	+	1-1	11	(0)	36	(4)	+	+	
B	+	1-1	2	(0)	8	(0)	-	+	
C	-	1-7	30	(1)	7	(1)	-	+	+
D	-	1-7	194	(7)	15	(0)	-	+	*mut8*
3A	+	1-1	1	(0)	10	(0)	-	+	+
B	-	1-7	236	(3)	9	(0)	+	+	*mut8*
C	-	1-7	249	(61)	16	(0)	-	+	*mut8*
D	+	1-1	0	(0)	8	(1)	-	+	+
4A	-	1-7	57	(2)	7	(0)	-	+	
B	+	1-1	2	(0)	7	(0)	-	+	
C	+	1-1	7	(0)	26	(0)	+	+	
D	-	1-7	199	(2)	12	(0)	+	+	*mut8*
5A	+	1-1	5	(0)	15	(0)	+	+	
B	-	1-7	337	(74)	13	(1)	-	+	*mut8*
C	+	1-1	0	(0)	2	(0)	+	+	
D	-	1-7	18	(0)	13	(0)	-	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 44 Phenotypes of spores recovered from cross RO409 ($\frac{mut7}{+}$ $\frac{his1\text{-}1}{his1\text{-}1}$)

Strain RO409	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	34°C	Designated mutator
1A	All are		0	(0)	51	(4)	-	-	*mut7*
B	*HOM+*,	*his1-1*	0	(0)	12	(0)	+	+	+
C			1	(0)	43	(0)	-	-	*mut7*
D			0	(0)	12	(0)	-	+	+
2A			0	(0)	13	(0)	+	+	+
B			1	(0)	8	(0)	+	+	+
C			4	(0)	32	(0)	-	-	*mut7*
D			1	(0)	44	(0)	-	-	*mut7*
3A			1	(0)	11	(0)	+	+	+
B			2	(0)	47	(1)	-	-	*mut7*
C			1	(0)	8	(0)	-	+	+
D			0	(0)	41	(0)	-	-	*mut7*
4A			1	(0)	13	(0)	+	+	+
B			1	(0)	34	(0)	-	-	*mut7*
C			0	(0)	16	(1)	+	+	+
D			0	(0)	73	(1)	-	-	*mut7*
5A			0	(0)	54	(15)	-	-	*mut7*
B			2	(0)	42	(0)	-	-	*mut7*
C			6	(0)	32	(0)	+/-	+	?
D			0	(0)	8	(0)	+	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 45: Phenotypes of spores recovered from cross RO410

($\frac{MUT+}{MUT+}$ $\frac{hom3\text{-}10\ his1\text{-}7\ +}{+\quad +\quad his1\text{-}1}$)

Strain RO410-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Survival at 36°C	Survival at 34°C	Designated mutator
1A	+	1-1	1	(0)	9	(0)	-	+	All are
B	+	1-1	0	(0)	9	(0)	+	+	*MUT* +
C	-	1-7	11	(0)	8	(0)	-	+	
D	-	1-7	10	(0)	12	(0)	+	+	
2A	+	1-1	0	(0)	10	(0)	-	+	
B	+	+			12	(0)	+	+	
C	-	1-7	7	(1)	6	(1)	-	+	
3A	-	1-7	5	(2)	9	(0)	+	+	
B	+	1-1	0	(0)	8	(0)	+	+	
C	+	1-1	0	(0)	3	(0)	+	+	
D	-	1-7	14	(0)	8	(1)	+	+	
4A	-	1-7	11	(1)	11	(0)	-	+	
B	-	1-7	6	(2)	7	(0)	+	+	
C	+	1-1	0	(0)	10	(0)	+	+	
D	+	1-1	1	(0)	7	(0)	+	+	
5A	-	1-7	15	(6)	11	(0)	-	+	
B	-	1-7	13	(0)	5	(0)	+	+	
C	+	1-1	0	(0)	12	(1)	+	+	
D	+	1-1	0	(0)	10	(0)	+	+	

** Numbers of prototrophs arising prior to growth on limiting medium are parenthesized.

TABLE 46: Phenotypes of spores recovered from cross RO411

$$\left(\frac{mut7}{mut7}\ \frac{hom3\text{-}10\ his1\text{-}7}{+\quad +}\ \frac{+}{his1\text{-}1}\right)$$

Strain RO411-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Designated mutator†
1A	+	1-1	3	(0)	58	(1)	All are *mut7*
B	+	1-1	0	(0)	44	(2)	
C	-	1-7	29	(12)	44	(1)	
D	-	1-7	28	(16)	42	(1)	
2A	-	1-7	59	(32)	52	(0)	
B	+	1-1	0	(0)	54	(2)	
C	+	1-1	3	(0)	41	(0)	
D	-	1-7	36	(20)	56	(0)	
3A	+	1-1	1	(0)	43	(0)	
B	-	1-7	49	(23)	34	(1)	
C	-	1-7	67	(13)	40	(0)	
D	+	1-1	1	(1)	57	(0)	
4A	+	1-1	2	(0)	63	(0)	
B	-	1-7	9	(9)	24	(0)	
C	+	1-1	1	(0)	56	(0)	
D	-	1-7	45	(11)	33	(0)	
5A	-	1-7	23	(12)	*	(1)	
B	+	1-1	0	(0)	60	(0)	
C	+	1-1	2	(0)	68	(2)	
D	-	1-7	145	(18)	52	(1)	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† All strains failed to grow at 34°C or 36°C.

* Plate contaminated.

TABLE 47: Phenotypes of spores recovered from cross RO417 ($\frac{MUT^+}{MUT^+}$)

Strain RO417-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Growth at 36°C†	Segregating alleles at *cry1*	Segregating alleles at *ade2*
1A	+	1-1	1	(0)	14	(0)	+	+	-
B	-	1-7	25	(0)	10	(0)	-	-	+
C	-	1-7	35	(0)	8	(0)	-	+	+
D	+	1-1	0	(0)	10	(0)	+	-	-
2A	-	1-7	30	(0)	12	(0)	+	+	+
B	+	1-1	0	(0)	5	(0)	+	+	-
C	+	1-1	0	(0)	8	(1)	-	-	-
D	-	1-7	20	(2)	17	(0)	+	-	+
3A	+	1-1	2	(0)	14	(0)	+	+	+
B	+	1-1	1	(0)	13	(0)	-	-	-
C	-	1-7	26	(2)	9	(2)	+	+	+
D	-	1-7	26	(0)	11	(0)	-	-	-
4A	+	1-1	0	(0)	14	(0)	+	-	+
B	-	1-7	28	(0)	7	(0)	-	+	+
C	-	1-7	63	(0)	11	(0)	+	-	-
D	+	1-1	3	(0)	13	(0)	-	+	-
5A	+	1-1	0	(0)	7	(0)	-	-	-
B	-	1-7	56	(2)	18	(0)	+	+	+
C	-	1-7	17	(0)	16	(1)	+	-	+
D	+	1-1	0	(0)	11	(0)	+	+	-
6A	-	1-7	45	(0)	13	(2)	+	+	-
B	-	1-7	14	(0)	12	(3)	+	+	+
C	+	1-1	0	(0)	4	(0)	-	-	-
D	-	1-7	46	(0)	11	(2)	-	-	+
7A	+	1-1	0	(1)*	9	(0)	+	+	+
B	-	1-7	45	(1)	12	(0)	+	+	+
C	-	1-7	30	(0)	13	(0)	+/-	-	-
D	+	1-1	1	(0)	9	(0)	-	-	-
8A	-	1-7	34	(24)	5	(0)	+	+	-
B	+	1-1	1	(0)	21	(0)	-	+	-
C	-	1-7	10	(0)	13	(0)	+	-	+
D	+	1-1	1	(0)	8	(0)	-	-	+
9A	-	1-7	14	(0)	8	(1)	+	+	+
B	+	1-1	1	(0)	7	(0)	+/-	+	+
C	+	1-1	1	(0)	9	(0)	-	-	-
D	-	1-7	17	(1)	17	(0)	+	-	-

TABLE 47: (continued)

Strain RO417-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Growth at 36°C†	Segregating alleles at *cry1*	*ade2*
11A	-	1-7	34	(0)	9	(0)	+	+	+
B	+	1-1	1	(0)	13	(0)	+	-	-
C	+	1-1	1	(0)	15	(0)	+	+	-
D	-	1-7	27	(0)	7	(0)	-	-	+
12A	+	1-1	0	(0)	12	(0)	-	+	-
B	-	1-7	47	(0)	8	(0)	+	+	+
C	+	1-1	0	(1)*	10	(0)	+	-	-
D	-	1-7	20	(0)	13	(0)	+	-	+
13A	+	1-1	3	(0)	11	(0)	+	+	-
B	-	1-7	16	(0)	23	(0)	+/-	-	-
C	+	1-1	1	(0)	12	(0)	+	+	+
D	-	1-7	20	(0)	8	(0)	+/-	-	+
14A	+	1-1	2	(0)	7	(0)	+/-	+	+
B	+	1-1	2	(0)	13	(0)	-	-	-
C	-	1-7	18	(0)	6	(0)	+	-	+
D	-	1-7	26	(0)	21	(0)	+	+	-

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† All spores grew at 34°C.

TABLE 48 : Phenotypes of spores recovered from cross RO428($\frac{mut7}{+}$)

Strain RO428-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Growth at 36°C†	Designated mutator
1A	-	1-7	58	(8)	46	(2)	-	*mut7*
B	+	1-1	0	(0)	10	(0)	+	+
C	-	1-7	19	(0)	11	(7)	+	+
D	+	1-1	2	(0)	41	(0)	-	*mut7*
2A	-	1-7	31	(4)	40	(0)	-	*mut7*
B	+	1-1	1	(0)	43	(0)	-	*mut7*
C	+	1-1	1	(0)	8	(0)	+	+
D	-	1-7	12	(3)	9	(0)	+	+
3A	-	1-7	29	(13)	50	(2)	-	*mut7*
B	+	1-1	3	(0)	16	(0)	+	+
C	-	1-7	13	(9)	53	(0)	-	*mut7*
D	+	1-1	2	(0)	12	(1)	+	+
*6A	-	1-7	15	(0)	4	(3)	+	+
B	+	1-1	0	(0)	28	(0)	-	*mut7*
C	-	1-7	47	(26)	66	(0)	-	*mut7*
D	+	1-1	1	(0)	13	(0)	+	+
7A	+	1-1	0	(0)	11	(0)	+	+
B	-	1-7	48	(7)	31	(0)	-	*mut7*
C	-	1-7	15	(2)	10	(0)	+	+
D	+	1-1	2	(0)	66	(0)	-	*mut7*
15A	+	1-1	2	(0)	66	(0)	-	*mut7*
B	-	1-7	31	(43)	43	(0)	-	*mut7*
C	+	1-1	0	(0)	14	(0)	+	+
D	-	1-7	15	(0)	14	(0)	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† The pattern shown here was identical to that observed at 34°C.

* Recombination between a/α & cry^r/cry^s resulted in the *mut7* strains 6B and 6C having a and α mating types while both being cry^s. These strains have been used as *mut7* 'testers' and are further described in table 2.

c. Spontaneous Prototrophs in

$\frac{hom3 \quad his1\text{-}7 \quad +}{+ \qquad + \; his1\text{-}1}$, $\frac{his1\text{-}7}{his1\text{-}7}$ and $\frac{his1\text{-}1}{his1\text{-}1}$ Strains

As noted in Table 33, heteroallelism at *his1* appeared to cause a five to ten-fold enhancement in Lassie scores. Since *his1-1* came to be present in *MUT*$^+$, *mut7*, and *mut8* backgrounds in the RO400 series of crosses (Table 2), the diploid configurations shown above could be tested extensively. Table 49 shows the results of the first analysis. In all cases *lys1-1* was homozygous and its reversion may be used for comparison of the effects of the *mut7* or *mut8* loci between strains. Previous unpublished work (Quah) as well as that shown here indicates that the two *mut* alleles are recessive for mutator activity--they cause little enhancement of homozygous *lys1-1* or *his1-7* reversion, even if both mutators are present in the heterozygous state in the same strain.

Strains RO402 and RO409 reverted minimally on limiting histidine as expected of homozygous *his1-1* strains (Fogel *et al.*, 1978). The *his1-7/his1-7* diploids RO405, 407 and 412 all had histidine Lassie scores of between fifteen and thirty revertants per plate. The heteroallelic *his1* strains RO403, 408 and 410 showed the same high reversion on limiting histidine as was noted in Table 33. Strain RO411, the *mut7* homozygote heteroallelic at *his1*, showed a large increase in histidine prototrophs compared to RO403, 408 and 410. Strain RO406, heterozygous for *mut7* and *mut8*, and heteroallelic at *his1* had an enhanced histidine Lassie

score, compared to the *MUT*$^+$ strains. A *mut8/mut8* diploid (bottom row) was included in the experiment to compare *mut8*-mediated *his1-7/his1-7* reversion.

In order to test the effect of *mut7 mut8* double mutant mutators on recombination at *his1*, strains derived from cross R088 (Tables 62 and 65) were crossed appropriately and tested using the Lassie protocol. *MUT*$^+$, *mut7* and *mut8* homozygotes were also tested. Table 50 shows the numbers of prototrophs observed on limiting histidine and lysine, as well as red lysine locus revertants, for *MUT*$^+$ homozygotes. For *mut7* homozygotes, the increased *his1* recombination noted in Table 49 was again observed (Table 51). Table 52 gives the Lassie scores of *mut8* homozygotes. Recombination appears to be reduced in *mut8/mut8*, *his1*-heteroallelic crosses, if contributions from *his1-7/his1-7* revertants are deducted. However, if one notes that the heteroallelic strains have only one *his1-7* allele, the apparent reduction is eliminated. For example, if the *MUT*$^+$ homozygote median recombination score, 164, is added to one-half of the *mut8* homozygotes' median *his1-7/his1-7* reversion score, 86, the value of 250 attained is close to the median number of prototrophs, 230, observed for the *his1-7/his1-1*, *mut8* homozygotes.

The apparent "additivity" of recombination and mutation suggests that *mut8*-mediated mutation occurs independently of intragenic recombination. This could not be demonstrated conclusively because the necessary *his1-7 his1-1* control was never isolated. Most histidine prototrophs were either $\frac{his1\text{-}7 \quad +}{+ \quad +}$ or $\frac{+ \quad +}{+ \; his1\text{-}1}$ (Tables 55 and 56), rather than the classically predicted

$\frac{his1\text{-}7\ his1\text{-}1}{+\quad +}$. This is in agreement with recent observations of mitotic, intragenic gene *conversion* occurring in the G1 stage of the cell cycle (Esposito, 1978). Furthermore, the specificity for conversion of only the *his1-1* allele shown by a MUT^+ homozygote (Table 57) casts doubt on the validity of the aforementioned algebraic method for estimating recombination at the *his1* locus.

Table 53 summarizes the data from Lassie tests of three different *mut7/mut7 mut8/mut8, his1-7/his1-1* diploids, plus controls. Again, the apparent loss of recombinants may be an artifact. The numbers observed fit the expected results if one-half of the homozygous *mut7 mut8*-mediated *his1-7/his1-7* median number of revertants are added to the median value obtained for *mut7/mut7*-mediated recombination. Note that *his1-1/his1-1* revertant frequency is greatly enhanced over that seen for MUT^+ homozygotes, as expected.

From Table 54 it can be seen that the *mut7* revertants *mut7-1-11* and *mut7-1-12* confer normal levels of recombination to diploids bearing them. This is true both when the revertants were crossed with *mut7-1*, and in the homozygous revertant diploid (*mut7-1-11/mut7-1-12*).

Histidine prototrophs which arose on limiting histidine were picked, retested on -his medium and sporulated, in the cases of RO516 and RO517 (Table 54). Three tetrads were picked from all sporulants (all isolates sporulated). All tetrads gave viable spores, although the *mut7/mut7* strain had many tetrads with two dead spores (Table 56).

The tetrads were then analysed to determine which *his1* allele had been converted to $HIS1^+$. Two spores from each set of tetrads which were not prototrophic for histidine were force-mated with *his1-7* or *his1-1* tester strains. The resulting diploids were tested for heteroallelism by UV-induction of *his1* prototrophs (see Savage, 1979, and Materials and Methods). Tables 55 and 56 describe the phenotypes of each histidine prototroph, picked from the Lassie plates, before and after sporulation. Four cross-feeding prototrophs were also picked from the Lassie plates per diploid, ostensibly as true mutation controls.

From Table 55 (columns 6 and 7) it can be seen that most spores from MUT^+ histidine prototrophs segregated 2 hom^-his^- : 2 HOM^+HIS^+, which suggests that the *his1-1* allele was the one more frequently converted to HIS^+. This is supported by the data shown in columns 9 and 10 of the same Table, that shows which his^- spores had (induced) intragenic recombination when force-mated to *his1-7* or to *his1-1* tester strains. Those diploids which were homoallelic rarely produced revertant clones following UV treatment of isolates spotted to -his plates ("-"). Heteroallelic diploids showed many revertants per streak ("+").

Table 56 suggests that the *mut7* homozygote produced a smaller proportion of *his1-1* → "+" convertants and more of the *his1-7* to HIS^+ than the MUT^+ strain. This may be seen more clearly in Table 57. Relative numbers of *his1-1* and *his1-7* convertants (first four rows) differed significantly between the *mut7* and the MUT^+ homozygotes ($\chi^2 = 3.97$, $P < 0.05$; corrected for continuity).

The origin of the cross-feeding revertants observed has been attributed to second site mutations in the *his1* gene by Fogel *et al.* (1978) and Lax and Fogel (1978). The cross-feeding prototroph #15 in Table 56 is reportedly due to a auxotrophic allele which complements the original *his1-7* mutation. However, one novel non-feeding revertant segregated 2:2 for a *his*$^-$ allele which complemented both *his1-1* and *his1-7* tester alleles for *growth* (Table 56, prototroph #11).

The putatively frameshift nature of *his1-1* may account for the specifity of *his1* conversion. The allele was crossed into all *mut* strains (section B) and may be considered the equivalent of the putative frameshift allele normally used to detect frameshift mutators in this laboratory, *hom3-10* (note that only *mut7 mut8* has been shown to confer greatly enhanced reversion of the *hom3-10* allele, of all the mutators tested (Quah, unpublished data)).

How does *mut7* affect meiotic, or mitotic intragenic recombination? The *mut7* homozygote RO413 (Table 2) which was heterozygous at *ade2* had 5/2793 red clones by direct plating, while the *mut7* heterozygotes RO415 and RO416, also *ade2-1/+* had no red segregants in a combined total of 5427 clones screened. It is unclear that *mut7* causes an enhancement in homozygosis because of the small numbers of red clones observed. Moreover, it was possible that the *ade2-1* allele may have been segregating meiotically; this possibility was not tested.

Meiotic intragenic recombination at *his1* appears to be unaltered in the *mut7/mut7* strain, RO430. 54/29200 *HIS*$^+$ prototrophs

were observed in sporulated diploids plated to -his medium, compared with 78/43700 for a *MUT*$^+$ homozygote, RO434.

It should be noted that the altered *his1* conversion seen in *mut7/mut7* strains was not corrected for the increased prototrophs observed in this strain. When this 3.8-fold increase in median Lassie score over the *MUT*$^+$ homozygote is considered, the proportionate numbers of *his1-7* and *his1-1* convertants for each strain change from those shown in Table 57 (first four rows) to the values seen below.

his1 allele converted	Number of prototrophs/class in *mut7* homozygotes	*MUT*$^+$ homozygotes
1-1	57	24
1-7	30.4	2

While the numbers still differ significantly, it is now evident that the *mut7* homozygote does *not* have reduced *his1-1* conversion relative to the control. Furthermore, the proportion of *his1-7* → *HIS*$^+$ prototrophs in *mut7* homozygotes (8/28) would give a frequency of 0.29, or 79/270 *his1-7* → *HIS*$^+$ prototrophs per plate (Tables 51, 54 and 74 give a value of ∿ 270 for *HIS*$^+$ prototrophs in the *mut7/mut7*, heteroallelic *his1* crosses). Seventy-nine *his1-7* prototrophs are considerably more than the numbers observed for *mut7*-mediated *his1-7/his1-7* reversion (Tables 51 and 74).

TABLE 49: Phenotypes of diploid strains constructed to study temperature sensitivities observed in *mut7* haploids, and heteroallelism at *his1* (experiment #2)

Diploid	Parents	Pertinant genotype	Growth at 36°C	Growth at 34°C	Prototrophs arising* on limiting histidine	Prototrophs arising* on limiting lysine
RO401	RO400-8A	*mut7* *his1-7*	+	+		12 (0)
	KF178-44D	+ +	+	+		18 (0)
			+	+		12 (0)
RO402	RO400-8B	*mut7* *his1-1*	+	+	1 (0)	18 (0)
	KF179-15A	+ *his1-1*	+	+	3 (0)	14 (0)
			+	+	2 (0)	23 (0)
RO403	RO400-8C	+ *his1-7* +	+	+	165 (8)	5 (0)
	KF179-15A	+ + *his1-1*	+	+	194 (25)	10 (0)
			+	+	185 (8)	11 (0)
RO404	RO400-8D	+ *his1-1*	+	+		14 (0)
	KF178-44D	+ +	+	+		18 (0)
			+	+		27 (0)
RO405	RO400-8A	*mut7* + *his1-7*	+	+	19 (2)	29 (0)
	XV731-10A	+ *mut8* *his1-7*	+	+	23 (0)	12 (0)
			+	+	18 (2)	14 (0)
RO406	RO400-8B	*mut7* + *his1-7* +	-	+	397 (23)	10 (0)
	XV731-3D	+ *mut8* + *his1-1*	-	+	348 (29)	11 (0)
			-	+	346 (21)	23 (0)
RO407	RO400-8C	+ *his1-7*	+	+	22 (0)	23 (0)
	XV731-3D	*mut8* *his1-7*	+	+	28 (0)	24 (0)
			+	+	20 (0)	17 (0)
RO408	RO400-8D	+ + *his1-1*	-	+	243 (15)	9 (0)
	XV731-10A	*mut8* *his1-7* +	-	+	199 (43)	13 (0)
			-	+	215 (6)	13 (1)
RO409	RO400-8B	*mut7* *his1-1*	-	+	0 (0)	8 (6)
	RO400-8D	+ *his1-1*	-	+	0 (0)	2 (0)
			-	+	0 (0)	17 (0)
RO410	RO400-8C	+ *his1-7* +	+	+	203 (29)	9 (1)
	RO400-8D	+ + *his1-1*	+	+	180 (40)	16 (0)
			+	+	203 (9)	11 (0)
RO411	RO400-8A	*mut7* *his1-7* +	-	-	520 (155)	52 (4)
	RO400-8B	*mut7* + *his1-1*	-	-	541 (105)	41 (4)
			-	-	577 (153)	52 (2)
RO412	RO400-8A	*mut7* *his1-7*	+/-	+	17 (1)	12 (2)
	RO400-8C	+ *his1-7*	+/-	+	21 (1)	19 (0)
			+/-	+	17 (0)	11 (1)
	XV731-3D	*mut8* *his1-7*	-	+	261 (55)	15 (2)
	XV731-10A	*mut8* *his1-7*	-	+	291 (48)	17 (0)
			-	+	256 (56)	27 (1)

* (See previous table)

TABLE 50: Spontaneous appearance of histidine or lysine prototrophs in *MUT*$^+$/*MUT*$^+$ diploid strains derived from cross RO88

Diploid	Parents	*his1* genotype	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants
RO441	RO88-4C / RO88-4D	+ / *1-7*	183	(5)	10	(0)	1
			169	(5)	10	(0)	1
			164	(6)	10	(0)	1
			140	(3)	13	(1)	1
RO446	RO88-14B / RO88-14A	+ / *1-7*	135	(17)	13	(2)	1
			167	(3)	16	(0)	2
			165	(20)	14	(0)	2
RO447	RO88-2D / RO88-9D	*1-7* / +	156	(78)	24	(0)	2
			170	(72)	22	(0)	1
			162	(58)	21	(0)	1
			129	(68)	24	(0)	2
RO442	RO88-5B / RO88-9D	+ / +	0	(0)	18	(0)	2
			<1	(0)	15	(0)	2
			0	(0)	9	(0)	1
			0	(0)	17	(0)	1
RO443	RO88-4C / RO88-9D	+ / +	<1	(0)	13	(0)	2
			0	(0)	16	(0)	0
			0	(0)	14	(0)	1
			<1	(0)	16	(0)	2
RO444	RO88-2D / RO88-4D	*1-7* / +	29	(1)	15	(4)	1
			27	(0)	13	(0)	1
			34	(1)	21	(0)	2
RO445	RO88-2D / RO88-8D	*1-7* / +	44	(0)	13	(0)	
			42	(1)	17	(0)	
			45	(1)	14	(0)	

** Numbers were averaged from three lassie plates. Prototrophs existing prior to growth on limiting medium are in parentheses.

TABLE 51: Spontaneous appearance of histidine or lysine prototrophs in *mut7/mut7* diploids derived from cross RO88

Diploid	Parents	*his1* genotype	Prototrophs arising** on limiting histidine		on limiting lysine		Red lysine revertants
RO448	RO88-1D / RO88-1B	*1-7 +* / *+ 1-1*	334	(42)	27	(0)	12
			281	(35)	30	(5)	16
			229	(42)	26	(1)	11
			278	(34)	26	(18)	12
			280	(17)	20	(34)	9
			278	(28)	28	(0)	10
			229	(19)	27	(0)	11
			247	(27)	26	(0)	13
RO449††	RO88-7C / RO88-8B	*1-7 +* / *+ 1-1*	226	(18)	25	(6)	8
RO453	RO428-6C / RO88-7C	*1-7 +* / *+ 1-1*	270	(42)	37	(0)	16
			308	(81)	49	(0)	15
RO452	RO88-8B / RO88-1B	*+ 1-1* / *+ 1-1*	<1	(0)	40	(12)	11
			<1	(0)	49	(0)	13
			<1	(0)	47	(0)	12
			<1	(0)	37	(0)	15
RO450	RO88-1D / RO88-7C	*1-7 +* / *1-7 +*	54	(0)	29†	(1)	7†
			45	(0)	23	(0)	11
			43	(0)	33	(1)	10
			49	(2)	73	(60)*	14

** Numbers were averaged from three lassie plates. Prototrophs existing prior to growth on limiting medium are in parentheses.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† Numbers were averaged from two plates.

†† Slow growing diploid strain; one isolate was counted after 14 days to ensure that *mut7/mut7* phenotypic levels of recombination occured.

TABLE 52: Spontaneous appearance of histidine or lysine prototrophs in *mut8/mut8* diploids derived from cross RO88

Diploid	Parents	*his1* genotype	Prototrophs arising** on limiting histidine		on limiting lysine		Red lysine revertants
RO454	RO88-1A / RO88-1C	*+ 1-1 / 1-7 +*	219	(14)	24	(0)	5
			196	(10)	19	(0)	6
			211	(12)	26	(0)	5
			214	(16)	24	(1)	3
			248	(6)	29	(0)	5
RO455	RO88-2A / RO88-7A	*1-7 + / + 1-1*	203	(69)	29	(0)	4
			240	(46)	26	(0)	1
			258	(22)	32	(0)	3
			250	(40)	20	(0)	5
			264	(27)	27	(0)	4
RO457	RO88-2A / RO88-1C	*1-7 + / 1-7 +*	210	(43)	21	(0)	
			177	(82)	28	(0)	
			151	(92)	21	(0)	
			164	(93)	25	(0)	
RO458	RO88-2A / RO88-6A	*1-7 + / 1-7 +*	190	(120)	27	(0)	2
			144	(155)	30	(0)	3
			177	(138)	23	(6)	1
			167	(136)	27	(0)	2
RO459	RO88-1A / RO88-7A	*+ 1-1 / + 1-1*	6	(0)	51	(42)*	3
			4	(0)	25	(0)	4
			5	(0)	24	(0)	3
			6	(0)	24	(0)	4

** Numbers were averaged from three lassie plates. Prototrophs existing prior to growth on limiting medium are in parentheses.

* 'Jackpot' (In these cases numbers not in parentheses include pre-existing prototrophs.)

TABLE 53: Spontaneous appearance of histidine or lysine prototrophs in *mut7*, *mut8*/*mut7*, *mut8* diploids derived from cross RO88

Diploid	Parents	*his1* genotype	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants
RO461	RO88-4B / RO88-4A	*1-7 +* / *+ 1-1*	804	(121)	80	(0)	43
			807	(140)	86	(0)	46
			639	(182)	79	(2)	45
			820	(161)	91	(2)	48
RO462	RO88-8C / RO88-10D	*+ 1-1* / *1-7 +*	279	(557)	99	(6)	
			664	(142)	68	(5)	
			834	(201)	78	(202)	
			744	(121)	101	(0)	
RO463	RO88-6B / RO88-9C	*+ 1-1* / *1-7 +*	834	(180)	69	(2)	49
			923	(96)	54	(0)	28
			798	(255)	65	(1)	45
			863	(244)	62	(0)	44
RO464	RO88-4B / RO88-10D	*1-7 +* / *1-7 +*	958	(182)	85	(0)	
			970	(191)	61	(0)	
			976	(93)	51	(18)	
RO466	RO88-6B / RO88-4A	*+ 1-1* / *+ 1-1*	35	(0)	78	(0)	50
			32	(0)	84	(0)	49
			33	(5)	81	(1)	48
RO467	RO88-8C / RO88-2B	*+ 1-1* / *+ 1-1*	37	(0)	74	(11)	37

** Numbers were averaged from three lassie plates. Prototrophs existing prior to growth on limiting medium are in parentheses.

TABLE 54 : Spontaneous appearance of histidine or lysine prototrophs in diploids heteroallelic at *his1*, and homozygous or heterozygous for *mut7-1*, or *mut7-1-11* or *mut7-1-12*, which are two ts. revertants of *mut7-1*

Diploid	Parents	Pertinent genotypes at *mut7*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants	Growth at 36°C
RO516	RO428-6B	*7-1*	*1-1 +*	321,292	(45)	41,29	(0)	19,11	-
	RO428-6C	*7-1*	*+ 1-7*	249,224	(153)	47,51	(1)	20,19	-
	RO428-6B	*7-1*	*1-1 +*	107,95	(30)	18,8	(2)	0,2	+
	RO428-6C^r	*7-1-12*	*+ 1-7*	70,85	(21)	13,25	(0)	0,2	+
				86,103	(32)	12,10	(6)	2,1	+
	RO428-6B^r	*7-1-11*	*1-1 +*	115,91	(26)	17,14	(0)	1,0	
	RO428-6C	*7-1*	*+ 1-7*	295,310	(254)*	13,16	(0)	0,2	+
				109,98	(25)	15,23	(0)	1,1	+
	RO428-6B^r	*7-1-11*	*1-1 +*	56,118	(37)	10,13	(0)	2,2	+
	RO428-6C^r	*7-1-12*	*+ 1-7*	288,220	(227)*	7,5	(0)	0,0	+
	RO428-6B^r	*7-1-11*	*1-1 +*	76,94	(6)	15,15	(0)	1,1	+
	RO400-10C	*+*	*+ 1-7*	75,82	(10)	15,6	(0)	2,2	+
	RO107-5C	*+*	*1-1 +*	85,71	(48)	14,9	(0)		+
	RO428-6C^r	*7-1-12*	*+ 1-7*	98,105	(50)	16,12	(0)		+
RO517	RO400-10C	*+*	*+ 1-7*	78,77	(1)	11,15	(0)		+
	RO107-5C	*+*	*1-1 +*	71,62	(10)	15,20	(17)		+

** Numbers of prototrophs existing prior to growth on limiting medium are in parentheses.
* 'Jackpot' (In these cases numbers not in parentheses include pre-existing prototrophs.)

TABLE 55: *his1* genotypes of diploid prototrophic clones from strain RO517 arising on limiting histidine medium

Initial Genotype : $\frac{ADE^+}{ADE^+}$ $\frac{hom3\text{-}10}{+}$ $\frac{his1\text{-}7}{+}$ $\frac{+}{his_4\text{-}1}$

Prototroph number	Diploid *hom3* phenotype	Spores recovered/ 3 tetrads	Number of spores analysed	Number of *his*⁻ spores observed	*hom3* phenotype *his*⁻ spores	*HIS*⁺ spores	*his*⁻ spores crossed to testers	>>25 UV-induced *HIS*⁺ clones/spot x*his1-7* tester	x*his1-1* tester	*his1* hetero-allele converted to *HIS*⁺
Plate #1 1	all +	12	12	6	+	-	2	+	-	1-7
2		9	7	4	-	+	in all	-	+	1-1
3		11	8	4	-	+	cases	-	+	1-1
4		11	8	4	-	+		-	+	1-1
5		12	12	6	-	+		-	+	1-1
6		12	12	6	-	+		-	+	1-1
7		12	12	6	-	+		-	+	1-1
8		8	8	4	-	+		-	+	1-1
9		9	8	4	-	+		-	+	1-1
10		12	12	6	-	+		-	+	1-1
11		12	12	6	-	+		-	+	1-1
12		12	12	6	-	+		-	+	1-1
13		11	8	4	+	-		+	-	1-7
'Feeder' 14		12	12	6	+	-		+	-	1-7S
'Feeder' 15		12	12	0		2+:2-				?
Plate #2 16		12	8	4	-	+		-	+	1-1
17		11	8	4	-†	+		-	+	1-1
18		12	8	4	-	+		-	+	1-1
19		12	8	4	-	+		-	+	1-1
20		10	8	3	-	+		-	+	1-1
21		12	8	4	-	+		-	+	1-1
22		10	8	4	-	+		-	+	1-1
23		11	8	4	-	+		-	+	1-1
24		12	8	4	+	-		-	+	1-1
25		8	8	4	-	+		-	+	1-1
26		12	8	4	-	+		-	+	1-1
27		9	8	4	-	+		-	+	1-1
28		12	8	4	-	+		-	+	1-1
'Feeder' 29		9	9	4	+	-		+	-	1-7S
'Feeder' 30		12	9	4	+	-		+	-	1-7S

* Strain was remated and retested. † One recombinant (tetratype) tetrad. See text for further explanation.

TABLE 56: *his1* genotypes of diploid prototrophic clones from strain RU516 arising on limiting histidine medium

Initial Genotype : $\frac{mus7\ hcm3\text{-}10\ his1\text{-}7\ +}{mus7\ +\ +\ his1\text{-}1}$

Prototroph Number	Diploid *hcm3* Phenotype	Spores recovered /3 tetrads	Number of spores analysed	Number of *his*⁻ spores observed	*hcm3* phenotype *his*⁻ spores	*hcm3* phenotype *HIS*⁺ spores	*his*⁻ spores crossed to testers	>>25 UV-induced *HIS*⁺ clones/spot *xhis1-7* tester	>>25 UV-induced *HIS*⁺ clones/spot *rhis1-1* tester	*his1* allele converted to *HIS*⁺
Plate #1 1	+	6	6	4	-	+	2	-	+	1-1
2	+	3	3	1	+	-	1	+	-	1-7
3	+	12	12	6	-	+	2	-	+	1-1
4	+	11	8	4	-	+	2	-	+	1-1
5	+	10	8	4	-	+	2	-	+	1-1
6	+	12	12	6	+	-	2	-	+	1-1
7	+	12	12	6	+	-	2	+	-	1-7
8	+	10	10	5	+	-	1	+	-	1-7
9	+	8	8	4	+	-	2	+	-*	1-7
10	+	12	12	6	-	+	2	-	+	1-1
11	+	12	8	4	+	-	2	+	-	1-7
12	+	7	7	3	-	+	2	**	**	?
13	-	8	8	4	-	-	2	-	+	1-1
'Feeder' 14	+	12	12	5	+	-	2	+	-	1-7S
'Feeder' 15	-	11	8	0	all -					?
Plate #2 16	+	9	9	5	-+	+	2	-	+	1-1
17	+	9	9	5	+	-	2	+	-	1-7
18	+	11	8	4	-	+	1	-	+	1-1
19	+	10	8	4	-	+	2	-	+	1-1
20	+	9	9	4	-	+	2	-	+	1-1
21	+	6	6	3	-	+	2	-	+	1-1
22	+	5	3	3	-	+	2	-	+	1-1
23	+	6	6	1	+	-	1	+	-*	1-7
24	+	10	8	4	-	+	2	-	-	1-1
25	+	12	12	6	+	-	2	+	-*	1-7
26	+	8	8	5	-	+	2	-	+	1-1
'Feeder' 27	+	12	12	6	+	-	2	+	-	1-7S
'Feeder' 28	+	5	5	1	+	-	1	+	-	1-7S

* Spore clones were remated and retested. ** *his*⁻ complemented both *his1* testers on -his. medium.
† One recombinant (tetratype) tetrad.

TABLE 57: *his1* genotypes of spontaneously arising histidine prototrophs derived from *mut7* (RO516) or *MUT*$^+$ (RO517) homozygous diploids heteroallelic at *his1*

Initial *his1* genotype : $\frac{hom3\text{-}10\ his1\text{-}7\ +}{+\ +\ his1\text{-}1}$

Presumptive genotype of histidine prototrophs	*his1* allele converted	Number of prototrophs/class in *mut7* homozygotes	Number of prototrophs/class in *MUT*$^+$ homozygotes	Isolate number of exceptional prototrophs/Strain
$\frac{hom3\text{-}10\ his1\text{-}7\ +}{+\ +\ +}$	*1-1*	13	23	
$\frac{hom3\text{-}10\ +\ +}{+\ +\ his1\text{-}1}$	*1-7*	8	2	
$\frac{hom3\text{-}10\ his1\text{-}7\ +}{hom3\text{-}10\ +\ +}$	*1-1*	1	0	#13 / RO516
$\frac{hom3\text{-}10\ +\ +}{+\ his1\text{-}7\ +}$	*1-1*	1	1	#6/RO516; #24/RO517
$\frac{hom3\text{-}10\ his1\text{-}7S\ +}{+\ +\ his1\text{-}1}$	Second site mutation	3	3	#14,27,28 / RO516 #14,29,30 / RO517
Other	?	2	1	#12,15 /RO516 #15 /RO517

B. Complementation, Allelism and Tetrad Analyses of Mutators Crossed to *mut7* or *mut8* Strains

1. Control Crosses of *mut7*, *mut8* and other single mutator strains

In order to test *mut7* and *mut8* for complementation and for segregation with respect to other mutator loci, it was necessary to determine:

(1) that other temperature sensitive mutations were not present,

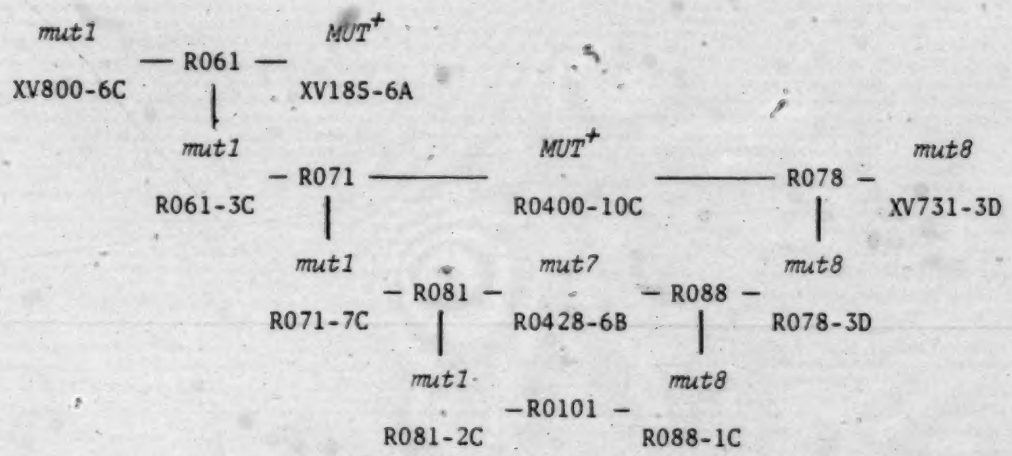
(2) that only one mutator locus was segregating,

(3) that *his1-7* and *lys1-1* were the only histidine and lysine requirements present, and

(4) the characteristic histidine and lysine Lassie scores for haploids and homozygous diploids of single mutator mutants in the mutators to be tested. (Tetrad analyses of crosses of *mut1/+* through *rad52/+* may be found in the appendix).

These criteria were also applied to the *mut7* and *mut8* tester strains employed. The genetics which produced the *mut7* tester strain R0428-6B were described in section 3. A suitable *mut8* strain was constructed in a manner similar to that employed for other mutator stocks (see Tables 3, 5, and Appendix, Table A1).

The following diagram summarizes crosses of *mut7* to *mut8* and of these loci to a given mutator locus, *mut1* in this case.



Segregating *mut7* or *mut8* spore clones provided internal controls in tetratype tetrads segregating for double mutator mutants from *mut7/+, +/mut1* or *mut8/+, +/mut1* heterozygotes. In addition, tetrad analyses of crosses involving the *mut7* tester strain R0428-6B and R088-1C have been included here for reference (Tables 58, 59, 60 and 61). A summary of Lassie scores from R088 spore clones was presented in Table 9. Numbers of red lysine (locus) lassie revertants found in R088, R0108 and R0428 spores are shown in Table 61.

Heterozygous mutator diploids of the R061 and R071 type shown in the above diagram were tested for mutator activity (Tables 62 and 63). Only *mut6/+* strains appeared to have enhanced (*lys1-1*) reversion. (Strains bearing *mut6*, *mut6/+* or *mut6/mut6* tended to become petite; petite derivatives of these strains had reduced mutator activity; see also Appendix, Table A1). Hence, care was taken to select for ρ^+ *mut6* strains, often by pregrowth on YG medium, prior to Lassie tests involving such strains.

TABLE 58: Phenotypes of spores recovered from cross RO88 ($\frac{mut7}{+}$ $\frac{+}{mut8}$)

Strain RO88-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Growth at 36°C	Designated mutator(s)
1A	+	1-1	2 (0)	19 (0)	+	+ *mut8*
B	+	1-1	2 (0)	29 (2)	-	*mut7* +
C	-	1-7	199 (58)	22 (2)	+	+ *mut8*
D	-	1-7	25 (21)	44 (0)	-	*mut7* +
2A	-	1-7	210 (86)	18 (0)	+	+ *mut8*
B	+	1-1	43 (0)	85 (1)	-	*mut7* *mut8*
C	+	1-1	0 (0)	24 (0)	-	*mut7* +
D	-	1-7	13 (0)	12 (0)	+	+ +
3A	+	1-7	11 (4)	14 (0)	+	+ +
B	-	1-7	437 (938)	131 (4)	-	*mut7* *mut8*
C	+	1-1	10 (2)	26 (0)	+	+ *mut8*
D	-	1-1	1 (0)	31 (0)	-	*mut7* +
4A	+	1-1	47 (0)	134 (2)	-	*mut7* *mut8*
B	-	1-7	807 (376)	83 (1)	-	*mut7* *mut8*
C	+	1-1	1 (0)	21 (26)	+	+ +
D	-	1-7	14 (0)	6 (0)	+	+ +
5A	-	1-7	559 (205)	92 (1)	-	*mut7* *mut8*
B	+	1-1	0 (0)	11 (0)	+	+ +
C	+	1-1	29 (0)	80 (2)	-	*mut7* *mut8*
D	-	1-7	62 (165)	34 (0)	+	+ *mut8*
6A	-	1-7	78 (243)	24 (0)	+	+ *mut8*
B	+	1-1	30 (6)	109 (0)	-	*mut7* *mut8*
C	+	1-1	0 (0)	48 (0)	-	*mut7* +
D	-	1-7	9 (0)	15 (0)	+	+ +
7A	-	1-1	3 (1)	30 (0)	+	+ *mut8*
B	+	1-7	650 (253)	112 (0)	-	*mut7* *mut8*
C	-	1-7	27 (3)	30 (0)	-	*mut7* +
8A	-	1-7	218 (65)	20 (1)	+	+ *mut8*
B	+	1-1	0 (0)	27 (0)	-	*mut7* +
C	+	1-1	26 (0)	123 (1)	-	*mut7* *mut8*
D	-	1-7	20 (3)	10 (0)	+	+ +
9A	-	1-7	72 (160)	29 (0)	+	+ *mut8*
B	+	1-1	2 (0)	22 (0)	-	*mut7* +
C	-	1-7	797 (241)	61 (0)	-	*mut7* *mut8*
D	+	1-1	1 (0)	16 (0)	+	+ +
10A	-	1-7	7 (4)	10 (0)	+	+ +
B	+	1-1	1 (0)	25 (8)	-	*mut7* +
C	+	1-1	8 (0)	23 (0)	+	+ *mut8*
D	-	1-7	1163 (1372)*	80 (14)	-	*mut7* *mut8*

TABLE 58: (continued)

Strain RO88-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Growth at 36°C	Designated mutator(s)	
11A	+	1-1?					+	+	
B	-	1-7					+	+	
C	-	1-7	27	(5)	42	(0)	-	*mut7*	
12A	-	1-7	572	(375)	105	(8)	-	*mut7*	*mut8*
B	+	1-1	1	(0)	40	(0)	-	*mut7*	+
13A	+	1-1	2	(0)	32	(0)	+	+	*mut8*
B	-	1-7	25	(5)	64	(0)	-	*mut7*	+
C	-	1-7	26	(5)	31	(1)	-	*mut7*	+
14A	-	1-7	7	(1)	17	(0)	+	+	+
B	+	1-1	1	(0)	12	(0)	+	+	+
C	+	1-1	21	(0)	85	(0)	-	*mut7*	*mut8*
15A	-	1-7	791	(233)	65	(0)	-	*mut7*	*mut8*
B	-	1-7	111	(191)	23	(0)	+	+	*mut8*
C	+	1-1	1	(0)	9	(0)	+	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs

TABLE 59: Phenotypes of spores recovered from cross RO107($\frac{mut8}{+}$)

Strain RO107-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Designated mutator
1A	-	1-7	4	(1)	11	(3)	+
B	+	1-1	1	(1)	16	(1)	8
C	+	1-1	3	(0)	16	(0)	8
2A	+	1-1	1	(0)	22	(0)	8
B	-	1-7	149	(106)	15	(1)	8
C	+	1-1	0	(0)	10	(0)	+
D	-	1-7	12	(2)	16	(0)	+
3A	+	1-1	4	(0)	23	(1)	8
B	-	1-7	17	(0)	10	(0)	+
C	-	1-7	168	(31)	11	(0)	8
D	+	1-1	0	(0)	16	(0)	+
4A	-	1-7	148	(39)	22	(0)	8
B	-	1-7	12	(1)	10	(0)	+
C	+	1-1	0	(0)	11	(0)	+
D	+	1-1	2	(0)	21	(2)	8
5A	-	1-7	165	(41)	20	(0)	8
B	+	1-1	0	(0)	12	(0)	+
C	+	1-1	0	(0)	12	(0)	+
D	-	1-7	184	(118)	30	(0)	8
6A	+	1-1	0	(0)	15	(0)	+
B	-	1-7	173	(41)	16	(0)	8
C	+	1-1	0	(0)	13	(0)	+
D	-	1-7	158	(72)	97	(123)*	8
7A	+	1-1	0	(0)	10	(0)	+
B	-	1-7	13	(0)	9	(0)	+
C	+	1-1	4	(0)	19	(0)	8
D	-	1-7	139	(174)	19	(0)	8
8A	+	1-1	0	(0)	12	(0)	+
B	+		73	(58)*	19	(0)	
C	-	1-7	14	(1)	13	(0)	+
D	-	1-7	147	(68)	21	(0)	8
9A	+	1-1	5	(0)	13	(0)	8
B	+	1-1	5	(0)	15	(1)	8
C	-	1-7	14	(0)	21	(0)	+
D	-	1-7	15	(2)	17	(0)	+
10A	-	1-7	16	(4)	7	(0)	+
B	+	1-1	0	(0)	9	(0)	+
C	-	1-7	184	(97)	25	(0)	8
D	+	1-1	3	(0)	23	(0)	8

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. * 'Jackpot'(In these cases unparenthesized numbers include pre-existing prototrophs.

TABLE 60: Phenotypes of spores recovered from cross RO108 ($\frac{mut8}{mut8}$)

Strain RO108-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Designated mutator
1A	+	1-1	5	(0)	19	(0)	All are *mut8-1*
B	+	1-1	2	(0)	19	(1)	
C	-	1-7	114	(155)	20	(0)	
D	-	1-7	178	(43)	21	(0)	
2A	+	1-1	4	(0)	20	(0)	
B	-	1-7	170	(61)	17	(0)	
C	+	1-1	5	(1)	29	(2)	
D	-	1-7	164	(43)	12	(0)	
3A	+	1-1	7	(1)	19	(0)	
B	-	1-7	161	(107)	16	(0)	
C	+	1-1	3	(0)	19	(1)	
D	-	1-7	167	(28)	22	(0)	
4A	+	1-1	2	(0)	11	(2)	
B	-	1-7	159	(57)	20	(0)	
C	+	1-1	4	(0)	14	(0)	
D	-	1-7	187	(109)	27	(0)	
5A	+	1-1	5	(0)	23	(0)	
B	-	1-7	201	(28)	14	(3)	
C	-	1-7	197	(45)	11	(1)	
D	+	1-1	6	(0)	24	(0)	
6A	-	1-7	175	(12)	23	(0)	
B	-	1-7	135	(43)	25	(0)	
C	+	1-1	7	(0)	23	(0)	
D	+	1-1	6	(0)	24	(0)	
7A	+	1-1	3	(0)	32	(0)	
B	-	1-7	144	(30)	17	(0)	
C	-	1-7	174	(78)	18	(0)	
D	+	1-1	2	(0)	15	(0)	
8A	+	1-1	7	(0)	20	(0)	
B	-	1-7	168	(14)	21	(0)	
C	+	1-1	5	(0)	21	(0)	
D	-	1-7	136	(65)	21	(0)	
9A	-	1-7	185	(30)	18	(0)	
B	+	1-1	3	(0)	13	(0)	
C	-	1-7	141	(71)	23	(1)	
D	+	1-1	5	(0)	21	(0)	
10A	-	1-7	162	(47)	24	(0)	
B	-	1-7	160	(60)	25	(0)	
C	+	1-1	3	(0)	18	(0)	
D	+	1-1	6	(0)	30	(1)	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 61: Presumptive *lys1-1* locus (red) revertants arising on limiting lysine in haploid strains bearing *mut7* and/or *mut8*

Strain RO428-	Designated mutator	Red Revertants
1A	*mut7*	15
B	+	0
C	+	0
D	*mut7*	18
2A	*mut7*	15
B	*mut7*	10
C	+	0
D	+	0
3A	*mut7*	19
B	+	0
C	*mut7*	22
D	+	0
6A	+	0
B	*mut7*	9
C	*mut7*	31
D	+	0
7A	+	1
B	*mut7*	7
C	+	1
D	*mut7*	20
15A	*mut7*	22
B	*mut7*	19
C	+	2
D	+	0

Strain RO88-	Designated mutator	Red Revertants
1A	+ *mut8*	5
B	*mut7* +	20
2B	*mut7* *mut8*	56
C	*mut7* +	9
3C	+ *mut8*	3
D	*mut7* +	7
4A	*mut7* *mut8*	87
5C	*mut7* *mut8*	51
D	+ *mut8*	3
6A	+ *mut8*	3
8B	*mut7* +	7
9C	*mut7* *mut8*	24
D	+ +	1
10A	+ +	1
C	+ *mut8*	4
13B	*mut7* +	21
14B	+ +	1
15B	+ *mut8*	6
RO108-		
1A	*mut8*	4
C	"	4
2A	"	5
D	"	3
3A	"	4
D	"	4
4B	"	3
D	"	3
5A	"	7
C	"	3
6C	"	9
D	"	4
7A	"	7
B	"	2
8B	"	7
C	"	4
9A	"	1
C	"	2
10B	"	5
C	"	6
D	"	7

2. Complementation Tests

Table 64 summarizes complementation tests of *mut7* with other mutator loci. Crosses R0430-R0436 (Table 65) were tested simultaneously to provide comparable *mut7/mut7*, *mut7/+* and MUT^+/MUT^+ Lassie scores. The diploids all complemented for temperature sensitivity and for Lassie scores, with the exception of the *mut7/+, +/mut5* heterozygote R085, which appeared to have reduced numbers of HIS^+ recombinants (all other scores overlapped those of the MUT^+/MUT^+ controls. *lys1-1* was used to assay for mutator activity, while *his1* recombination was assayed on limiting histidine.

Part of the complementation testing results for *mut8/+, +/mut* heterozygotes are presented in Table 66. While numbers of histidine prototrophs are enhanced, in some cases, over the results shown in Tables 64 and 65, this effect may be due either to heterozygosity for *mut8* (R0107, or see Table 75) or to fluctuations in Lassie scores between experiments. Again, the *mut5* heterozygote seems to have reduced histidine Lassie scores. The diploids had low levels of *lys1-1* reversion. However, it was impossible to ascertain whether any mutator complemented *mut8* for this phenotype because *mut8/mut8* mediated lysine Lassie scores are characteristically low.

Table 67 describes complementation tests for *rad51, rad52, mut6* and *mut9* with *mut7*, and for *mut6, mut9* and *rad52* with *mut8*. All strains grew at 36°C, were not γ-radiation sensitive, and had low levels of lysine reversion. The *rad51* and *rad52* heterozygotes had reduced spontaneous histidine prototrophy, but the presence of jackpots in the *mut7/+, +/rad52* strain prevented this conclusion from being drawn in the case of R094.

Tables 68 to 77 describe portions of one experiment designed to examine recombination more extensively in mutator strains, to complete complementation testing of *mut8* with other mutators and to provide an index of Lassie scores in mutator diploids. These results are summarized in Table 78.

Homozygous *his1-1* strains were not tested, because it was assumed that reversion at this locus would be minimal and hence would not contribute to Lassie scores in *his1* heteroallelic strains other than those homozygous for *mut8* (but see Table 53 for *his1-1* conversion).

Only *mut5* and *mut7* appear to confer altered recombination as detected by Lassie tests. *mut5/mut5* and *mut5/+* diploids produced the lowest median Lassie scores of the *his1* heteroallelic strains. Interestingly enough, the *mut9* homozygotes appeared to be enhanced for recombination. Unfortunately, no homozygous *his1-7, mut9* strains were available that were not contaminated by a second *mut8* allele. This allele was first detected segregating in spore clones from cross R089. Table 76 shows that the enhancement of mutation in crosses of *mut8-2* to *mut8-1* appears to occur without the presence of *mut9*. Table 79 confirms that the *mut8-2* allele does not complement with *mut8-1*, and that *mut8-2* appears to be the dominant allele as shown by the reduced *his1-7* reversion frequencies. This discovery explained the segregation of a mutator activity for *his1-7* from γ-radiation sensitive, *mut9* spore clones (Appendix, Table A1).

MUT^+/MUT^+ diploids were found to be consistent for *his1* recombination, even if the contributing strains were not closely related (Table 77). The slight reductions seen in R0468 and R0469 may have

been due to the failure of these strains to express crossfeeder revertants on limiting histidine. This result was unexpected. Further collection of data concerning the expression of feeders in diploids on Lassie tests is summarized in the following chart. Note that all haploids shown below expressed crossfeeders when tested (only two of the haploid strains studied, T1a and T1α (donated by L. Savage), have failed to do so). "+" indicates that feeders were observed.

Strains		α R0400-10C *his1-7*	R0122-1C *his1-7*	LZ13-1A *his1-1*
R0105-1A	*his1-7*	+	-	-
R0122-2C	*his1-7*	-	+	-
LZ13-2C	*his1-1*	-	-	- ?
KF179-15A	*his1-1*	+	-	- ?
R0428-6B^{r}11	*his1-1*	+	-	+
R0105-2B	*his1-1*	+	-	+

Absence of feeders in homozygous *his1-1* crosses may be due to the small numbers of prototrophs screened. A question mark is beside both of the cases listed. Otherwise, it would seem that certain diploids are complementing for a non-feeder phenotype. It would be interesting to know whether or not this trait is a function of repair.

Two other observations from this experiment should be noted here. Strains heterozygous for *mut5* appear to be dominant for MMS sensitivity. All *mut6* heterozygotes were recessive for *his1-7* reversion even when also heterozygous for *mut8* (approximately one-half of the *mut6/mut6* and *mut6/+* zygotes formed petite colonies; these were discarded).

TABLE 62: Phenotypes of diploids heterozygous for *mut1--mut9*, compared to homozygous *mut7/mut7* or *mut8/mut8* strains

Diploid	Parents	Pertinent Genotype	Prototrophs arising on limiting histidine**		Prototrophs arising on limiting lysine**	
RO60	RO1-90A	*mut7* *his1-7*	17	(2)	34	(0)
	XV732-2B	*mut7* *his1-7*	23	(4)	22	(0)
			13	(4)	33	(0)
RO47	XV731-3D	*mut8* *his1-7*	144	(11)	21	(0)
	XV731-10A	*mut8* *his1-7*	167	(12)	8	(1)
			134	(13)	25	(48)
RO58	RO1-90A	*mut7* + *his1-7*	7	(0)	12	(0)
	XV731-3D	+ *mut8* *his1-7*	12	(1)	9	(0)
			11	(0)	15	(1)
RO71	RO51-4D	*mut7* *mut8* *his1-7*	27		18	(0)
	XV185-6A	+ + *his1-7*	8		9	(0)
RO61	XV800-6C	*mut1* *his1-7*	19	(0)	64	(1)
	XV185-6A	+ *his1-7*			21	(0)
			21	(1)	66	(0)
RO62	XV354-2C	*mut2* *his1-7*	4	(0)	25	(0)
	XV185-6A	+ *his1-7*	8	(1)	19	(0)
RO63	XV195-24C	*mut3* *his1-7*	5	(0)	16	(0)
	XV185-6A	+ *his1-7*	4	(1)	5	(0)
			5	(1)	10	(1)
RO64	XV357-12D	*mut4* + *his5*			39	(0)
	XV185-6A	+ *his1-7* +			41	(0)
RO65	XV407-19D	*mut5* + *his5*			27	(1)
	XV185-6A	+ *his1-7* +			22	(0)
					22	(0)
					17	(0)
RO66	XV374-28C	*mut6* *his1-7*	5	(1)	2	(0)
	XV185-6A	+ *his1-7*	8	(0)	55	(0)
			4	(0)	26	(0)
RO67	XV731-2B	*mut7* *his1-7*	12	(0)	5	(0)
	XV185-6A	+ *his1-7*	12	(0)	9	(0)
			7	(0)	21	(0)
RO68	XV731-3D	*mut8* *his1-7*	13	(0)	19	(0)
	XV185-6A	+ *his1-7*	14	(1)	16	(0)
			7	(2)	10	(0)
RO69	XV396-1A	*mut9* *his1-7*	14	(0)	14	(0)
	XV185-6A	+ *his1-7*	12	(0)	10	(0)
			14	(1)	11	(1)

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. Strains were incubated on limiting medium for eight days.

TABLE 63: Phenotypes of diploids heterozygous for *mut1*--*mut9*, excepting *mut7*, crossed to RO400-10C

Diploid	Parents	Pertinent Genotype		Prototrophs arising** on limiting histidine		lysine	
RO71	RO61-3C	*mut1*	*his1-7*	11	(1)	9	(5)
	RO400-10C	+	*his1-7*	3	(3)	25	(1)
				16	(15)*	33	(0)
RO72	RO62-2A	*mut2*	"	14	(0)	11	(6)
	RO400-10C	+		7	(0)	25	(0)
				8	(0)	22	(0)
RO73	RO63-5C	*mut3*	"	4	(1)	15	(11)
	RO400-10C	+		9	(1)	13	(0)
RO74	RO64-3D	*mut4*	"	9	(0)	25	(0)
	RO400-10C	+		12	(0)	11	(0)
				16	(1)	12	(3)
RO75	RO65-5A	*mut5*	"	15	(7)	28	(0)
	RO400-10C	+		39	(3)	17	(0)
RO76	RO66-6B	*mut6*	"	13	(1)	64	(1)
	RO400-10C	+		10	(1)	61	(7)
				20	(0)	30	(4)
RO78	XV731-3D	*mut8*	"	25	(0)	10	(0)
	RO400-10C	+		30	(1)	12	(1)
				20	(4)	19	(1)
RO79	RO69-2D	*mut9*	"	24	(11)	8	(0)
	RO40 -10C	+		19	(0)	15	(5)

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. Strains were incubated for six days after pre-growing zygotes on YG medium.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 64: Phenotypes of diploids heterozygous for *mut7* and one other *mut* locus, and heteroallelic at *his1*

$$\left(\frac{mut7}{+}\ \frac{+}{mut}\ \frac{+}{hom3\text{-}10}\ \frac{+}{his1\text{-}7}\ \frac{his1\text{-}1}{+}\right)$$

Diploid Strain	Parents	*mut* genotype	Growth at 36°C	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants
RO81	RO71-7C	+ *mut1*	+	90	(12)	20	(3)	0
	RO428-6B	*mut7* +	+	90	(10)	20	(0)	0
			+	106	(18)	27	(0)	0
			+	80	(23)	26	(1)	3
RO82	RO72-1C	+ *mut2*	+	70	(25)	12	(0)	1
	RO428-6B	*mut7* +	+	80	(12)	13	(0)	1
			+	62	(19)	15	(0)	0
			+	95	(18)	13	(0)	0
RO83	RO73-5D	+ *mut3*	+	297	(279)*	11	(0)	2
	RO428-6B	*mut7* +	+	218	(210)*	14	(0)	0
			+	314	(335)*	9	(0)	0
			+	101	(85)	9	(0)	0
RO84	RO74-3A	+ *mut4*	+	80	(6)	9	(0)	0
	RO428-6B	*mut7* +	+	57	(19)	15	(0)	0
			+	68	(15)	30	(30)*	1
			+	49	(35)	15	(0)	0
RO85	RO75-4B	+ *mut5*	+	55	(8)	9	(15)	1
	RO428-6B	*mut7* +	+	41	(9)	14	(0)	0
			+	39	(9)	10	(0)	0
RO86	RO76-6D	+ *mut6*	+/-	106	(47)	188	(149)*	1
	RO428-6B	*mut7* +	+/-	131	(19)	12	(0)	0 †
RO88	RO78-3D	+ *mut8*	+	84	(39)	12	(1)	0
	RO428-6B	*mut7* +	+	90	(49)	10	(0)	1
			+	89	(40)	8	(0)	1
			+	114	(41)	15	(1)	0
RO89	RO79-8A	+ *mut9*	+	80	(121)	11	(0)	1
	RO428-6B	*mut7* +	+	92	(91)	12	(0)	2
			+	103	(58)	13	(0)	1
			+	75	(103)	16	(0)	1

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. All numbers are the average of two determinations.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† This strain failed to grow when replica-plated to YG medium.

For *mut7/mut7* and mut^+/mut^+ controls, see Table 65.

TABLE 65: Phenotypes of diploids constructed for studying heteroallelism at *his1* in *mut7/mut7* and *mut+/mut+* strains (experiment #3)

Diploid	Parents	Pertinant genotype†	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Red lysine revertants
RO430	RO428-6C / RO428-6B	*mut7* / *mut7* *his1-7 +* / *+ his1-1*	193 (207)	39 (2)	17
			187 (198)	45 (1)	19
			710 (693)*	34 (0)	16
			233 (151)	43 (1)	15
			230 (140)	42 (3)	17
RO431	RO428-6B / RO428-7D	*mut7* / *mut7* *his1-1* / *his1-1*	1 (0)	40 (14)	20
			1 (0)	46 (0)	15
			0 (0)	45 (1)	18
			1 (0)	35 (1)	20
RO432	RO428-6C / RO428-1A	*mut7* / *mut7* *his1-7* / *his1-7*	17 (1)	51 (0)	19
			30 (5)	34 (0)	14
			23 (1)	47 (10)	22
			15 (6)	41 (21)	21
RO433	RO428-7D / RO428-3C	*mut7* / *mut7* *his1-7 +* / *+ his1-1*	242 (175)	36 (3)	18
			187 (173)	35 (1)	14
			227 (78)	42 (2)	14
RO434	RO428-1C / RO428-1B	*mut+* / *mut+* *his1-7 +* / *+ his1-1*	108 (71)	61 (54)*	2
			114 (33)	130 (156)*	0
			133 (17)	8 (1)	0
			97 (26)	14 (0)	0
RO435	RO428-1B / RO428-2D	*mut+* / *mut+* *+ his1-1* / *his1-7 +*	79 (28)	9 (0)	1
			87 (52)	9 (0)	1
			85 (27)	14 (0)	1
RO436	RO428-15D / RO428-1C	*mut+* / *mut+* *his1-7* / *his1-7*	5 (1)	17 (0)	
			3 (1)	19 (0)	
			7 (1)	14 (1)	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases pre-existing prototrophs are included in the unparenthesized numbers.)

† *mut7/mut7* strains are first tested for failure to grow at 36°C.

TABLE 66: Phenotypes of diploids heterozygous for *mut8* and one other *mut* locus, and heteroallelic at *his1*

$$\left(\frac{mut8}{+}\ \frac{+}{mut}\ \frac{hom3\text{-}10\ his1\text{-}7}{+}\ \frac{+}{+}\ \frac{+}{his1\text{-}1}\right)$$

Diploid	Parents	*mut* genotype	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Red lysine revertants
RO108	RO88-1A / RO88-1C	*mut8* / *mut8*	190 (27)	16 (0)	4
			189 (14)	17 (0)	3
			245 (10)	25 (0)	5
RO107	RO88-4C / RO88-1C	+ / *mut8*	126 (17)	18 (0)	
			140 (8)	8 (0)	
			121 (13)	18 (0)	
RO101	RO81-2C / RO88-1C	+ *mut1* / *mut8* +	176 (47)	32 (0)	
			120 (70)	26 (0)	
RO102	RO82-6D / RO88-1C	+ *mut2* / *mut8* +	156 (10)	18 (0)	1
			178 (8)	16 (0)	1
			143 (28)	17 (0)	1
RO103	RO83-6C / RO88-1C	+ *mut3* / *mut8* +	107 (24)	22 (0)	2
			120 (29)	17 (0)	1
RO104	RO84-4C / RO88-1C	+ *mut4* / *mut8* +	160 (6)	14 (1)	
			163 (12)	12 (0)	
			153 (9)	20 (0)	
RO105	RO85-17A / RO88-1C	+ *mut5* / *mut8* +	95 (20)	17 (0)	2
			91 (4)	14 (0)	2
RO106	RO86-21C / RO88-1C	+ *mut6* / *mut8* +	135 (18)	27 (0)	1
			199 (35)	61 (0)	1
			164 (43)	56 (0)	2
RO109	RO89-2D / RO88-1C	+ *mut9* / *mut8* +	197 (23)	16 (0)	0
			174 (20)	27 (0)	2
			160 (18)	26 (0)	0

** Numbers were averaged from two 'lassie' plates. Prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 67 : Phenotypes of diploids heterozygous for *mut7* or *mut8* and one other *mut* locus, and heteroallelic at *his1*

Diploid	Parents	*mut* loci	*his1* genotype	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Red lysine revertants
RO99	RO400-10C / RO428-6B	+ / *mut7*	*his1-7* + / + *his1-1*	85 (2)	11 (0)	0
				105 (19)	11 (0)	1
				103 (5)	17 (0)	1
RO100	RO88-2D / RO428-6B	+ / *mut7*	*his1-7* + / + *his1-1*	160 (54)	19 (0)	1
				143 (67)	20 (0)	1
				116 (57)	22 (1)	1
RO93	RO91-1C / RO428-6B	+ *51* / *7* +	*his1-7* + / + *his1-1*	99 (12)	13 (0)	
				99 (4)	10 (0)	
				85 (4)	19 (0)	
RO94	RO92-6B / RO428-6B	+ *52* / *7* +	*his1-7* + / + *his1-1*	265 (261)*	12 (1)	1
				197 (154)*	10 (0)	1
†				478 (482)*	9 (0)	1 †
RO506	RO106-4C / RO428-6B	+ *6* / *7* +	*his1-7* + / + *his1-1*	109 (9)	37 (0)	0
				103 (0)	42 (0)	1
				98 (6)	147 (151)	0
RO507	RO89-2D / RO88-13C	+ *9* / *7* +	+ *his1-1* / *his1-7* +	130 (2)	15 (0)	1
				159 (5)	20 (0)	2
				128 (1)	22 (11)	1
RO512	RO106-4C / RO104-5A	+ *6* / *8* +	*his1-7* + / + *his1-1*	99 (4)	24 (0)	
				90 (6)	28 (0)	
				90 (3)	26 (0)	
RO109	RO89-2D / RO88-1C	+ *9* / *8* +	+ *his1-1* / *his1-7* +	118 (5)	20 (0)	2
				133 (12)	23 (0)	0
				120 (4)	25 (0)	0
RO111	RO94-2A / RO88-1A	+ *52* / *8* +	*his1-7* + / + *his1-1*	61 (2)	14 (0)	2
				75 (10)	16 (0)	2
				78 (5)	10 (0)	1

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. Numbers were averaged from two lassie plates.

* 'Jackpot (In these cases unparenthesized numbers include pre-existing prototrophs.)

† RO506-RO111 is a separate experiment. The control in this case is RO109, which has been lassied previously (see Table 66).

TABLE 68 : Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut1**

Diploid	Parents	Pertinent genotypes *mut*	*his1*	Prototrophs arising** on limiting histidine		lysine		Red lysine revertants
RO131	RO71-7C	*mut1*	*1-7 +*	43,40	(2)	124,177	(25)	
	RO71-1D	*mut1*	*1-7 +*	38,38	(1)	139,109	(1)	
				44,31	(3)	408,290	(692)	
RO141	RO71-1D	*mut1*	*1-7 +*	105,105	(14)	135,141	(0)	
	RO81-2C	*mut1*	*+ 1-1*	137,123	(11)	147,136	(31)	
				119,107	(5)	58,93	(161)	
RO171	RO71-7C	*mut1*	*1-7 +*	5,8	(1)	21,25	(0)	1,1
	RO105-1A	+	*1-7 +*	3,9	(0)	29,32	(0)	1,0
RO181	RO71-7C	*mut1*	*1-7 +*	70,69	(14)	13,14	(0)	0,1
	RO105-2B	+	*+ 1-1*	73,93	(6)	22,23	(0)	0,1
RO191	RO71-7C	*mut1* +	*1-7 +*	15,8	(3)			
	RO78-8D	+ *mut8*	*1-7 +*	17,16	(2)			
				17,24	(22)			

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* Tables 68-77 present data from the same experiment.

TABLE 69: Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut2*

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants	Growth on MMS
RO132	RO72-1C / RO72-6A	*mut2* / *mut2*	*1-7* + / *1-7* +	49,61	(4)	87,122	(0)		-
				67,77	(1)	115,142	(2)		-
				59,82	(12)	106,109	(0)		-
RO142	RO72-6A / RO82-6D	*mut2* / *mut2*	*1-7* + / + *1-1*	159,124	(30)	97,107	(1)	3,5	-
				159,130	(19)	94,93	(5)	3,2	-
				136,125	(12)	118,116	(48)	1,1	-
RO172	RO72-1C / RO105-1A	*mut2* / +	*1-7* + / *1-7* +	7,7	(1)	11,15	(0)	1,2	+
				1,9	(2)	21,15	(0)	2,3	+
RO182	RO72-1C / RO105-2B	*mut2* / +	*1-7* + / + *1-1*	58,58	(11)	11,11	(0)	1,2	+
				42,69	(12)	13,17	(0)	1,1	+
RO192	RO72-1C / RO78-8D	*mut2* + / + *mut8*	*1-7* + / *1-7* +	2,4	(10)				+
				13,13	(6)				+
				8,3	(8)				+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 70 : Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut3*

Diploid	Parents	Pertinent genotypes *mut*	*his1*	Prototrophs arising** on limiting histidine	lysine	Red lysine revertants	Growth on MMS
RO133	RO73-5D	*mut3*	*1-7 +*	41,54 (3)	31,20 (0)		-
	RO73-2A	*mut3*	*1-7 +*	53,41 (7)	22,46 (6)		-
				46,26 (0)	24,39 (1)		-
RO143	RO73-2A	*mut3*	*1-7 +*	77,85 (23)	32,24 (0)	1,3	-
	RO83-6C	*mut3*	*+ 1-1*	74,118 (31)	26,27 (0)	1,1	-
				92,88 (18)	26,29 (1)	1,3	-
RO173	RO73-5D	*mut3*	*1-7 +*	5,8 (1)	11,12 (0)	0,1	+
	RO105-1A	+	*1-7 +*	7,11 (0)	8,7 (0)	0,0	+
RO183	RO73-5D	*mut3*	*1-7 +*	79,68 (6)	8,8 (0)	0,2	+
	RO105-2B	+	*+ 1-1*	59,62 (6)	8,11 (0)	0,1	+
RO193	RO73-5D	*mut3* +	*1-7 +*	7,6 (3)			+
	RO78-8D	+ *mut8*	*1-7 +*	2,7 (8)			+
				6,7 (4)			+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 71 : Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut4*

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants	Growth on MMS
RO134	RO74-3A	*mut4*	*1-7* +	62,15	(42)	15,22	(3)		-
	RO74-1B	*mut4*	*1-7* +	43,41	(0)	22,13	(0)		-
				39,45	(22)	24,38	(0)		-
RO144	RO74-1B	*mut4*	*1-7* +	123,105	(189)	25,23	(3)		-
	RO84-4C	*mut4*	+ *1-1*	123,168	(11)	1426,1233	(1262)*		-
				115,113	(24)	31,22	(0)		-
RO174	RO74-3A	*mut4*	*1-7* +	6,6	(0)	10,13	(0)	0,1	+
	RO105-1A	+	*1-7* +	1,10	(0)	11,18	(0)	0,0	+
RO184	RO74-3A	*mut4*	*1-7* +	56,65	(4)	21,17	(0)	2,1	+
	RO105-2B	+	+ *1-1*	62,74	(18)	13,18	(0)	0,0	+
RO194	RO74-3A	*mut4* +	*1-7* +	11,3	(5)				+
	RO78-8D	+ *mut8*	*1-7* +	16,14	(6)				+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 72: Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut5*

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants	Growth on MMS	Growth after γ
RO135	RO75-4B	*mut5*	*1-7 +*	30,30	(1)	71,80	(0)		-	-
	RO75-1B	*mut5*	*1-7 +*	27,36	(3)	84,105	(7)		-	-
				317,292	(254)*	55,77	(2)		-	+ †
RO145	RO75-1B	*mut5*	*1-7 +*	28,36	(3)	67,66	(12)	1,1	-	-
	RO85-17A	*mut5*	*+ 1-1*	45,22	(0)	69,60	(87)	2,1	-	-
				30,22	(4)	78,102	(2)	2,4	-	-
RO175	RO75-4B	*mut5*	*1-7 +*	15,13	(0)	15,22	(1)	2,1	-	+
	RO105-1A	*+*	*1-7 +*	10,27	(0)	21,11	(2)	1,0	-	+
RO185	RO75-4B	*mut5*	*1-7 +*	46,63	(9)	17,18	(0)	0,0	-	+
	RO105-2B	*+*	*+ 1-1*	48,51	(8)	13,15	(0)	3,1	-	+
				65,76	(5)	21,16	(0)	3,2	-	+
RO195	RO75-4B	*mut5* / *+*	*1-7 +*	3,24	(6)				-	+
	RO78-8D	*+* / *mut8*	*1-7 +*	11,14	(12)				-	+
				19,19	(10)				-	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)
† This strain contains an apparent revertant of *mut5*.

TABLE 73: Spontaneous appearance of histidine or lysine Prototrophs in diploids containing *mut6*

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants
RO136	RO76-6D / RO76-1C	*mut6* / *mut6*	*1-7 +* / *1-7 +*	22,25	(1)	33,40	(0)	
				47,43	(1)	48,58	(0)	
				20,16	(0)	24,43	(0)	
				30,35	(0)	65,63	(0)	
RO146	RO76-1C / RO86-21C	*mut6* / *mut6*	*1-7 +* / *+ 1-1*	108,94	(18)	46,45	(2)	0,1
				152,111	(13)	46,48	(0)	1,0
RO176	RO76-6D / RO105-1A	*mut6* / *+*	*1-7 +* / *1-7 +*	9,7	(0)	31,42	(1)	0,1
				5,10	(0)	26,26	(0)	0,0
				7,9	(0)	29,32	(0)	0,1
RO186	RO76-6D / RO105-2B	*mut6* / *+*	*1-7 +* / *+ 1-1*	162,178	(39)	46,57	(0)	
RO196	RO76-6D / RO78-8D	*mut6* / *+*	*1-7 +* / *1-7 +*	6,26	(27)			
		+ / *mut8*		25,24	(2)			
				23,12	(2)			
				8,6	(4)			

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 74: Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut7*

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Red lysine revertants	Growth at 36°C	Growth on MMS
RO137	RO428-6C	*mut7*	*1-7 +*	12,16	(2)	42,44	(0)	13,9	-	-
	RO88-13C	*mut7*	*1-7 +*	27,24	(2)	43,54	(1)	14,13	-	-
				19,27	(0)	39,65	(3)	20,18	-	-
RO147	RO428-6C	*mut7*	*1-7 +*	245,299	(54)	31,34	(0)	9,11	-	-
	RO428-6B	*mut7*	*+ 1-1*	263,295	(66)	34,47	(0)	8,21	-	-
				253,262	(72)	27,33	(12)	10,19	-	-
RO177	RO428-6C	*mut7*	*1-7 +*	4,8	(0)	23,21	(1)	2,0	+	+
	RO105-1A	+	*1-7 +*	5,9	(0)	20,15	(0)	0,0	+	+
RO187	RO428-6C	*mut7*	*1-7 +*	77,88	(20)	12,13	(0)	3,0	+	+
	RO105-2B	+	*+ 1-1*	58,124	(25)	12,21	(0)	0,3	+	+
RO197	RO428-6C	*mut7* +	*1-7 +*	12,8	(6)				+	+
	RO78-8D	+ *mut8*	*1-7 +*	6,7	(4)				+	+
				3,8	(2)				+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 75: Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut8*, and serving as control strains for results shown in Tables 68-74

Diploid	Parents	Pertinent genotypes *mut*	Pertinent genotypes at *his1*	Prototrophs arising** on limiting histidine		lysine		Red lysine revertants
RO138	RO78-3D / RO78-8D	*mut8* / *mut8*	*1-7* + / *1-7* +	220,174	(221)	16,19	(0)	
				169,199	(217)	17,18	(0)	
				210,178	(217)	21,16	(1)	
RO178	RO78-3D / RO105-1A	*mut8* / +	*1-7* + / *1-7* +	13,5	(8)	21,23	(1)	0,0
				9,12	(3)	19,23	(0)	0,1
RO188	RO78-3D / RO105-2B	*mut8* / +	*1-7* + / + *1-1*	87,100	(8)	18,26	(0)	0,0
				76,106	(6)	16,25	(0)	2,1

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 76: Spontaneous appearance of histidine or lysine prototrophs in diploids containing *mut9*, and/or the presumptive *mut8* allele *mut8-2*

Diploid	Parents	Pertinent genotypes *mut9*	*mut8*	at *his1*	Prototrophs arising** on limiting histidine		lysine		Red lysine revertants	Growth on MMS	after γ
RO139	RO69-2D	*mut9*	*8-2*	*1-7 +*	134,82	(22)	61,82	(9)		-	-
	RO79-8A	*mut9*	*8-2*	*1-7 +*	75,88	(34)	75,79	(6)		-	-
					134,89	(29)	104,90	(117)		-	-
RO151	RO69-2D	*mut9*	*8-2*	*1-7 +*			377,350	(303)*	5,5	-	-
	RO89-6C	*mut9*	*8-2*	*1-7 +*			92,97	(12)	0,0	-	-
RO152	RO79-8A †	*mut9*	*8-2*	*1-7 +*			32,46	(23)	0,0	-	-
	RO508-2C	*mut9*	+	*1-7 +*			65,86	(6)	0,2	-	-
RO149	RO69-2D	*mut9*	*8-2*	*1-7 +*	177,202	(26)	94,83	(3)	3,1	-	-
	RO89-2D	*mut9*	+	*+ 1-1*	198,191	(28)	74,76	(4)	11,3	-	-
					218,224	(14)	82,94	(2)	7,2	-	-
RO153	RO69-2D	*mut9*	*8-2*	*1-7 +*	37,37	(6)	12,25	(1)	0,1	+	+/-
	RO514-2D	+	*8-2*	*1-7 +*	35,60	(7)	20,21	(0)	1,1	+	+/-
					37,39	(6)	22,14	(0)	1,0	+	+/-
					42,41	(29)	20,27	(0)	1,1	+	+
					41,61	(26)	16,11	(0)	3,0	+	+
RO179	RO79-8A	*mut9*	*8-2*	*1-7 +*	7,13	(2)	16,18	(0)	0,0	+	+
	RO105-1A	+	+	*1-7 +*	1,9	(5)	19,19	(1)	2,1	+	+
RO155	RO514-2D	+	*8-2*	*1-7 +*	9,5	(1)	16,19	(0)	0,1	+	+
	RO105-1A	+	+	*1-7 +*	8,9	(2)	13,20	(0)	1,0	+	+
RO189	RO79-8A	*mut9*	*8-2*	*1-7 +*	90,121	(2)	20,21	(0)	1,2	+	+
	RO105-2B	+	+	*+ 1-1*	102,128	(15)	15,17	(0)	0,2	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.
† This cross is also heterozygous for *mut7*.

TABLE 77: Spontaneous appearance of histidine or lysine prototrophs in *MUT*$^+$/*MUT*$^+$ diploids derived from various strains, and serving as control strains for results shown in Tables 68-76.

Diploid	Parents	*his1* genotype	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Red lysine revertants
RO129	RO400-10C / RO105-2B	*1-7 +* / *+ 1-1*	85,86 (6)	13,10 (0)	1,0
			64,68 (10)	16,9 (0)	0,0
			88,76 (5)	15,8 (0)	1,1
RO150	RO428-6C^r / RO428-6B^r	*1-7 +* / *+ 1-1*	95,82 (10)	9,16 (0)	1,0
			111,95 (11)	11,14 (0)	1,0
RO468	RO122-2C / LZ13-1A	*1-7 +* / *+ 1-1*	56,62 (4)		
			58,49 (7)		
RO469	RO122-1C / LZ13-2C	*1-7 +* / *+ 1-1*	68,83 (14)		
			88,76 (4)		
RO130	RO400-10C / RO105-1A	*1-7 +* / *1-7 +*	7,9 (1)	14,11 (0)	1,1
			2,4 (3)	9,13 (0)	3,0
			14,5 (0)	10,10 (0)	0,0
RO140	RO428-6C^r / RO105-1A	*1-7 +* / *1-7 +*	6,11 (0)	12,13 (0)	1,1
			7,9 (0)	12,9 (0)	0,0
			7,4 (0)	14,8 (0)	0,0

** Numbers of prototrophs existing prior to growth on limiting medium are parénthesized.

TABLE 78: A summary of the phenotypes of homozygous and heterozygous mutators (all tested in one experiment; Tables 68 to 77)

Mutator genotype	Median Lassie score *his1-7*/*his1-7*	*his1-7*/*his1-7*	*lys1-1*/*lys1-1* Total	*lys1-1*/*lys1-1* Locus	Strain viablity at 36°C	Strain viablity on MMS	Strain viablity after γ
mut1/*mut1*	113	39	138		+	+	+
mut2/*mut2*	133	64	108	3	+	-	+
mut3/*mut3*	87	44	27	1	+	-	+
mut4/*mut4*	119	41	22		+	-	+
mut5/*mut5*	29	30	71	2	+	-	-
mut6/*mut6*	111	28	46	1	+	+	+
mut7/*mut7*	263	22	41	13	-	-	+
mut8/*mut8*		189	18		+	+	+
mut9/*mut9**	200		76	2	+	-	-
mut1/ +	72	7	23	1	+	+	+
mut2/ +	58	7	14	1	+	+	+
mut3/ +	65	8	8	0†	+	+	+
mut4/ +	64	6	15	0	+	+	+
mut5/ +	57	14	16	1	+	-	+
mut6/ +	170**	8	32	0	+	+	+
mut7/ +	83	7	18	0	+	+	+
mut8/ +	94	11	22	0	+	+	+
mut9/ +*	112	8	19	1	+	+	+
MUT$^+$/*MUT*$^+$	79	7	12	0	+	+	+

* heterozygous for *mut8-2*

** only one isolate tested

† a "0" as opposed to a blank indicates that at least one locus revertant was noted in the strain tested but that the median score was less than 0.5

TABLE 79: Confirmation of the presence of an allele of *mut8* (*mut8-2*) in *mut9* strains by complimentation testing with *mut8-1* testers, using homozygous *his1-7/his1-7* diploids.

Diploid	Parents	*mut8* genotype	Prototrophs arising** on limiting histidine	lysine	Red lysine revertants
Experiment #1					
RO199 †	RO79-8A / RO78-8D	*+ 8-2* / *8-1 +*	94,98 (105)* 38,26 (87)		
RO156 †	RO79-8A / RO514-2A	*+ 8-2* / *8-1 + ?*	49,8 (74) 50,36 (46)	10,13 (1) 9,17 (0)	0,1 1,5
RO157 †	RO514-2A / RO78-8D	*8-1 + ?* / *+ 8-2*	62,42 (99) 29,49 (58) 143,125 (125)		
Experiment #2					
	XV731-3D / XV731-10A	*8-1 +* / *8-1 +*	162,212 (15) 196,190 (30) 201,192 (13)		
	RO69-1B / RO69-3A	*+ 8-2* / *+ 8-2*	36,45 (3)		
	RO69-1B / XV731-3D	*+ 8-2* / *8-1 +*	59,44 (13) 87,63 (21)		
	RO69-3A / XV731-10A	*+ 8-2* / *8-1 +*	84,83 (11) 63,72 (23)		
	RO400-10C / XV731-3D	*+ +* / *8-1 +*	13,19 (4) 3,10 (5) 20,17 (1)		
	RO428-15D / XV731-10A	*+ +* / *8-1 +*	13,15 (2) 14,15 (1)		
	RO69-1B / RO428-15D	*+ 8-2* / *+ +*	9,3 (2) 12,11 (0) 10,17 (2)		
	RO69-3A / RO400-10C	*+ 8-2* / *+ +*	17,21 (3)		
	RO400-10C / RO428-15D	*+ +* / *+ +*	5,9 (0) 1,4 (2)		
	RO400-10C / RO105-1A	*+ +* / *+ +*	10,6 (1) 5,11 (0) 11,13 (0)		

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. * "Jackpot" (In these cases unparenthesized numbers include pre-existing prototrophs.) † These diploids are also heterozygous for *mut9* (for controls see Table 77).

3. Tetrad Analyses of Mutators Crossed to the *mut7* and *mut8* Strains

At least ten tetrads from each cross of *mut7* or *mut8* to *mut1* through *mut9* (and *rad52*) were dissected. These were screened by replica-plating or spot test for auxotrophies, mating type, UV, γ and MMS sensitivities where applicable, as well as for mutator activity. Tetrads from crosses heterozygous *only* for *mut1* through *mut9* and *rad52* were analysed prior to making the above crosses (the RO60- and RO70-series) and the results are shown in the appendix (Tables A1a to j) for comparison. Where double mutator mutants have segregated (i.e. *mut7 mut1*), most have been confirmed by outcrossing to *mut* tester strains. (These data are found in Appendix Tables A2a-g). Two tetratype [++, *mut*+, +*mut*, *mut mut*] tetrads from each cross were further tested, where possible, to determine whether differences in Lassie scores between spores might be due to different final cell numbers per lassie plate in the double mutator. This was not the case except for crosses involving *mut2*.

The mutators *mut1*, *mut2*, *mut3* and *mut4* have been characterized by Gottlieb and von Borstel (1976), and von Borstel and Quah (unpublished data) for *lys1-1* mutation. I include references to their data when comparing these loci.

a. *mut1* (Tables 80, 81 and 82)

The *mut1* strains are characterized by a twenty- to thirty-fold increase in *lys1-1* suppressor reversion rates; *lys1-1* locus reversion rates appear to be reduced (Gottlieb and von Borstel, 1976), while *his1-7* reversion rates increase three- to four-fold over *MUT*$^+$ strains.

When *mut1* and *mut7* are present in the same strain, *mut1* should be detectable because the strain should be highly revertable for *lys1-1* (or other suppressible markers). The *mut7* locus can be scored by its temperature sensitivity, while *mut8* should be ascertainable by its enhancement of *his1-7* or *his1-1* reversion, and by the "*mut8* effect" of stationary phase mutator activity for *his1-7* reversion.

Mutator loci appear to assort independently, from both of the $\frac{mut7}{+}\ \frac{+}{mut1}$ (Table 80) and $\frac{mut8}{+}\ \frac{+}{mut1}$ (Table 81) heterozygotes. (1P:7T:2N in RO81; OP:4T:1N in RO101). The *mut1* strains do not have enhanced *his1-1* reversion (for example, see tetrad #8, Table 80).

Table 82a gives the mean revertants per plate for the mutator strains from RO81 and RO101. There was no significant difference between means, either for MUT^+ or for *mut1* spores from these two crosses, and so both experiments have been grouped together. In this case, and for some of the other crosses, *his1-1* reversion data were omitted because only spores segregating for *mut8* had significant numbers of revertants at this test locus. The *mut8 mut1 his1-1* and *mut8 his1-1* spores shown in Table 85 had similar histidine lassie scores. Table 82b gives the spontaneous mutation rate, M, in terms of background cell growth, for histidine and lysine reversion in some strains from Tables 80 and 81. (The second page of Table 80 is a repetition in order to obtain enough data to calculate standard errors.) As can be seen from both Tables 82a and 82b, the *mut1 mut7* strains produced, respectively, lysine Lassie scores and ratios that were more than additive with respect to *mut1* and *mut7* strains, while histidine reversion approached addivitity.

TABLE 80: Phenotypes of spores recovered from cross RO81($\frac{mut7}{+}$ $\frac{+}{mut}$)

Strain RO81-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Growth at 36°C	Designated mutator(s)	
2A	-	1-7	74	(0)	391	(18)	-	*mut7*	*mut1*
B	-	1-7	50	(10)	26	(0)	-	*mut7*	+
C	+	1-1	0	(0)	254	(4)	+	+	*mut1*
D	+	1-1	0	(0)	9	(0)	+	+	+
3A	-	1-7	10	(2)	19	(0)	+	+	+
B	+	1-1	0	(0)	34	(0)	-	*mut7*	+
C	-	1-7	96	(9)	331	(213)	-	*mut7*	*mut1*
D	+	1-1	1	(0)	248	(120)	+	+	*mut1*
4A	-	1-7	7	(1)	12	(0)	+	+	+
B	+	1-1	0	(0)	12	(0)	+	+	+
C	-	1-7	136	(11)	1591	(1223)*	-	*mut7*	*mut1*
5A	-	1-7	77	(10)	40	(0)	-	*mut7*	+
B	-	1-7	12	(0)	11	(1)	+	+	+
C	+	1-1	0	(0)	623	(471)*	-	*mut7*	*mut1*
D	+	1-1	0	(0)	373	(13)	+	+	*mut1*
6A	+	1-1	1	(0)	416	(1)	-	*mut7*	*mut1*
B	-	1-7	71	(11)	39	(1)	-	*mut7*	+
C	-	1-7	75	(6)	378	(9)	+	+	*mut1*
D	-	1-1	0	(0)	10	(0)	+	+	+
7A	+	1-1	0	(0)	12	(0)	+	+	+
B	-	1-7	115	(7)	264	(6)	-	*mut7*	*mut1*
C	-	1-7	39	(3)	24	(0)	-	*mut7*	+
D	+	1-1	0	(0)	346	(7)	+	+	*mut1*
8A	-	1-7	23	(12)	18	(0)	-	*mut7*	+
B	+	1-1	0	(0)	264	(32)	+	+	*mut1*
C	-	1-7	36	(3)	39	(1)	-	*mut7*	+
D	+	1-1	1	(0)	458	(87)	+	+	*mut1*
9A	+	1-1	0	(0)	7	(0)	+	+	+
B	-	1-7	106	(3)	314	(454)	-	*mut7*	*mut1*
C	-	1-7	7	(2)	9	(0)	+	+	+
D	+	1-1	0	(0)	444	(16)	-	*mut7*	*mut1*
10A	-	1-7	56	(4)	53	(0)	-	*mut7*	+
B	+	1-1	0	(0)	414	(28)	-	*mut7*	*mut1*
C	-	1-7	63	(3)	234	(4)	+	+	*mut1*
D	+	1-1	0	(0)	15	(0)	+	+	+
11A	-	1-7	134	(6)	441	(6)	-	*mut7*	*mut1*
B	-	1-7	16	(0)	7	(4)	+	+	+
C	+	1-1	1	(0)	254	(3)	+	+	*mut1*
D	+	1-1	2	(0)	71	(1)	-	*mut7*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 80: (continued)

Experiment #2

Strain RO81-	Prototrophs arising** on limiting histidine		lysine		Designated mutator(s)	
2A	88	(2)	391	(43)	?	*1*
B	55	(2)	43	(1)	?	+
C	0	(0)	298	(104)	+	*1*
D	1	(0)	14	(0)	+	+
3A	13	(1)	11	(0)	+	+
B	0	(0)	45	(0)	?	+
C	117	(7)	487	(2)	?	*1*
D	0	(0)	255	(67)	+	*1*
4A	31	(1)	15	(0)	+	+
B	0	(0)	15	(0)	+	+
C	122	(4)	555	(1)	?	*1*
5A	90	(32)	47	(0)	?	+
B	30	(0)	14	(1)	+	+
C	1	(0)	495	(32)	?	*1*
D	0	(0)	250	(581)	+	*1*
6A	1	(0)	342	(5)	?	*1*
B	123	(10)	40	(0)	?	+
C	105	(2)	387	(0)	+	*1*
D	0	(0)	78	(74)*	+	+
7A	0	(0)	15	(0)	+	+
B	147	(5)	725	(658)*	?	*1*
C	65	(1)	40	(0)	?	+
D	0	(0)	274	(281)	+	*1*
8A	54	(25)	23	(0)	?	+
B	0	(0)	1315	(1220)*	+	*1*
C	46	(11)	46	(1)	?	+
D	0	(0)	388	(98)	+	*1*
9A	0	(0)	10	(0)	+	+
B	129	(83)	443	(96)	?	*1*
C	32	(1)	18	(0)	+	+
D	0	(0)	601	(17)	?	*1*
10A	39	(6)	55	(0)	?	+
B	0	(0)	344	(312)	?	*1*
C	65	(2)	311	(39)	+	*1*
D	0	(0)	12	(0)	+	+
11A	110	(14)	353	(120)	?	*1*
B	7	(0)	9	(0)	+	+
C	0	(0)	309	(2)	+	*1*
D	0	(0)	68	(2)	?	+

*, ** see previous page.

TABLE 81: Phenotypes of spores recovered from cross R0101($\frac{mut8}{+}$ $\frac{+}{mut1}$)

Strain R0101-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Designated mutator(s)	
1A	+	1-1	0,0	(0)	402,347	(1)	+	*mut1*
B	+	1-1	3,6	(0)	439,339	(6)	*mut8*	*mut1*
C	-	1-7	16,17	(0)	9,5	(0)	+	+
2A	-	1-7	10,12	(0)	13,12	(0)	+	+
B	-	1-7	65,69	(2)	315,289	(428)	+	*mut1*
C	+	1-1	4,3	(0)	29,16	(0)	*mut8*	+
D	+	1-1	7,4	(0)	454,437	(19)	*mut8*	*mut1*
3A	-	1-7	11,6	(0)	21,17	(0)	+	+
B	+	1-1	5,3	(0)	434,330	(3)	*mut8*	*mut1*
C	+	1-1	4,3	(0)	399,419	(10)	*mut8*	*mut1*
D	-	1-7	10,13	(1)	9,20	(0)	+	+
4A	+	1-1	2,0	(0)	295,281	(3)	+	*mut1*
B	+	1-1	4,6	(0)	28,26	(0)	*mut8*	+
5A	+	1-1	0,1	(0)	367,262	(7)	+	*mut1*
B	-	1-7	120,94	(17)	14,27	(0)	*mut8*	+
6A	+	1-1	0,1	(0)	246,202	(14)	+	*mut1*
B	+	1-1	1,3	(0)	25,14	(0)		+
C	-	1-7	204	(26)	16,23	(0)	*mut8*	+
D	-	1-7	103	(2)	232,299	(8)		*mut1*
7A	-	1-7	79	(3)	284	(71)	+	*mut1*
B	+	1-1	3,8	(0)	17,31	(0)	*mut8*	+
C	-	1-7	318,360	(72)	357	(8)	*mut8*	*mut1*
D	+	1-1	0,0	(0)	8,14	(0)	+	+
8A	-	1-7	59,50	(22)	366,358	(7)	+	*mut1*
B	-	1-7	209,263	(103)	431,485	(3)	*mut8*	*mut1*
C†	+	1-1	7,5	(0)	22,16	(0)	*mut8*	+
D†	+	1-1	7,2	(0)	20,14	(0)	*mut8*	+
10A†	+	1-1	2,0	(0)	324,251	(1)	+	*mut1*
B†	-	1-7	315,202	(229)	486,455	(1)	*mut8*	*mut1*
C†	-	1-7	10,11	(0)	17,8	(0)	+	+
D†	+	1-1	6,12	(0)	27,19	(0)	*mut8*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† Histidine lassie data is from a separate experiment (controls were identical in the two experiments).

TABLE 82a: Summary of Lassie scores for crosses RO81 and RO101

his1-7 reversion	Mutator(s)					
	MUT$^+$	*mut1*	*mut7*	*mut8*	*mut1mut7*	*mut1mut8*
Mean	14.1	70	59	139	115	278
S.E.	1.8	5.5	7	33	6.5	26
lys1-1 reversion						
Mean	12.4	308	42	22	413	420
S.E.	0.7	11	3.5	1.5	22	15

b: Mutation rates (M) in terms of (unreverted) cells per Lassie plate of strains from crosses RO81 and RO101

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/plug * x 10^{-4}	M_{lys} x 10^{8}	Histidine Lassie score*	Cells/plug * x 10^{-4}	M_{his} x 10^{8}
mut1	81-6C	1-7	354	241§	62	79	143§	23
	101-7A	"	278	206§	57	87	165§	22
	81-3A	1-1	252 (1)	202§	52	1	115§	0.4
	101-6A	"				1	143§	0.3
mut7	81-6B	1-7	38	177	9.0	96	130	31
	-3B	1-1	47 (21)	196	10	1	131	0.2
mut8	101-6C	1-7	14 (4)	181	3.2	164	142	48
	-7B	1-1	20 (10)	201	4.2	7	177	1.7
mut1, *mut7*	81-3C	1-7	422 (1)	188§	94	121	113§	45
	-6A	1-1	286 (1)	149§	80	1	131§	0.8
mut1, *mut8*	101-7C	1-7	351	256§	57	272	160§	71
	-3B	1-1	323 (1)	215§	63	3	156§	0.8
MUT$^+$	81-3A	1-7	14	154	3.8	12	152	3.3
	101-3A	"	19	188	4.2	20	128	6.5

* Average of two determinations
§ Average of four determinations
Numbers in parentheses are the number of red (locus) revertants/plate

Additivity cannot be ruled out for *mut1 mut8* reversion for either *his1-7* or *lys1-1*.

The numbers of cells on lysine Lassie plates are higher in haploids derived from RO81 and RO101 than for haploids from other crosses. This may account for the elevated lysine Lassie scores for *mut7*, *mut8* and *MUT*$^+$ in these strains (see data for *mut2* crosses for comparison).

b. *mut2* (Tables 83, 84 and 85)

Most *mut2* strains show a five to ten-fold enhancement over *MUT*$^+$ strains for both *lys1-1* and *his1-7* reversions. The *lys1-1* locus revertants may be slightly enhanced (Table 85). The *mut2* strains may be detected by a non-*ts* MMS-sensitive phenotype (Nasim and Brychcy, 1979). The *mut7* and *mut8* phenotypes were scored according to the characteristics mentioned in the section describing the *mut1* locus. The *mut2* allele assorts independently from *mut7* (1P:6T:3N) and from *mut8* (1P:8T:0N). The *mut2* mutation does not significantly enhance *his1-1* reversion, whether alone, or as *mut2 mut7* (Table 83) or *mut2 mut8* (Table 84), over scores seen for *MUT*$^+$ strains.

In Table 89a the mean Lassie scores for *his1-7* suggest that both *mut7* and *mut8* interact less than additively with *mut2* for reversion of this allele. The *mut7 mut2* and *mut8 mut2* strains were additive over the respective single mutators for *lys1-1*. The situation appears to be totally reversed in Table 9b. These data (from a separate experiment) suggest that both double mutators are additive for *his1-7* reversion, while *mut7 mut2* strains may be additively enhanced for

TABLE 83: Phenotypes of spores recovered from cross R082 ($\frac{mut7}{+} \times \frac{+}{mut2}$)

Strain R082-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival on MMS	Designated Mutator(s)	
1A	-	1-7	73 (9)	93 (1)	+	-	+	*mut2*
B	+	1-1	4 (0)	121 (203)	-	-	*mut7*	*mut2*
C	+	1-1	0 (0)	40 (0)	-	-	*mut7*	+
D	-	1-7	14 (1)	7 (0)	+	+	+	+
2A	+	1-1	0 (0)	7 (1)	+	+	+	+
B	-	1-7	33 (1)	31 (13)	-	-	*mut7*	+
C	+	1-1	0 (0)	133 (19)	-	-	*mut7*	*mut2*
3A	+	1-7	6 (0)	12 (0)	+	+	+	+
B	-	1-1	0 (0)	11 (0)	+	+	+	+
C	+	1-1	2 (0)	96 (65)	-	-	*mut7*	*mut2*
4A	+	1-1	0 (0)	42 (1)	-	-	*mut7*	+
B	-	1-7	82 (9)	78 (35)	+	-	+	*mut2*
C	-	1-7	67 (4)	73 (18)	+	-	+	*mut2*
D	+	1-1	0 (0)	69 (0)	-	-	*mut7*	+
5A	-	1-7	85 (6)	83 (0)	-	-	*mut7*	*mut2*
B	+	1-1	4 (0)	122 (0)	+	-	+	*mut2*
C	-	1-7	65 (5)	32 (0)	-	-	*mut7*	+
6A	-	1-7	51 (2)	41 (0)	-	-	*mut7*	+
B	+	1-1	5 (0)	103 (2)	-	-	*mut7*	*mut2*
C	-	1-7	3 (3)*	15 (1)	+	+	+	+
D	+	1-1	0 (0)	82 (1)	+	-	+	*mut2*
7A	+	1-1	1 (0)	93 (18)	-	-	*mut7*	*mut2*
B	-	1-7	162 (120)*	205 (186)*	-	-	*mut7*	*mut2*
C	-	1-7	9 (4)	15 (0)	+	+	+	+
D	+	1-1	1 (0)	15 (0)	+	+	+	+
8A	+	1-1	0 (0)	13 (0)	+	+	+	+
B	-	1-7	107 (5)	151 (2)	-	-	*mut7*	*mut2*
C	-	1-7	33 (1)	46 (2)	-	-	*mut7*	+
D	+	1-1	2 (0)	74 (66)	+	-	+	*mut2*
9A	-	1-7	6 (0)	14 (1)	+	+	+	+
B	-	1-7	6 (0)	17 (0)	+	+	+	+
C	+	1-1	2 (0)	161 (110)*	-	-	*mut7*	*mut2*
10A	+	1-1	1 (0)	15 (0)	+	+	+	+
B	-	1-7	767(1060)*	97 (1)	-	-	*mut7*	*mut2*
C	+	1-1	2 (0)	97 (2)	+	-	+	*mut2*
D	-	1-7	45 (15)	119 (141)*	-	-	*mut7*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs

TABLE 84 Phenotypes of spores recovered from cross RO102($\frac{mut8}{+}$ $\frac{+}{mut2}$)

Strain RO102-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth on MMS	Designated mutator(s)
1A	-	1-1	0,0 (0)	4,6 (0)	+	+ +
B	+	1-1	2,3 (0)	80,80 (39)	-	*mut8* *mut2*
C	-	1-7	144,162(140)	22,18 (1)	+	*mut8* +
D	+	1-7	98,83 (8)	102,88 (24)	-	+ *mut2*
2A	-	1-7	107,96 (0)	67,85 (12)	-	+ *mut2*
B	+	1-1	5,8 (0)	118,82 (1)	-	*mut8* *mut2*
C	+	1-1	1,0 (0)	11,6 (0)	+	+ +
D	-	1-7	124,72 (95)	25,21 (0)	+	*mut8* +
3A	+	1-1	3,5 (0)	72,52 (2)	-	*mut2*
B	-	1-7	259,346(108)	52,113(162)	-	*mut2*
C	-	1-7	142,186(65)	17,16 (0)	+	*mut8* +
D	+	1-1	2,0 (0)	12,13 (0)	+	+ +
4A	+	1-1	0,1 (0)	7,13 (0)	+	+ +
B	-	1-7	146,181(100)	24,17 (0)	+	*mut8* +
C	+	1-1	4,1 (0)	806,765(685)*	-	+ *mut2*
D	-	1-7	140,155(373)	72,98 (1)	-	*mut8* *mut2*
5A	-	1-7	102,99 (80)	27,23 (0)	+	*mut8* +
B	-	1-7	15,12 (1)	17,10 (0)	+	+ +
6A	+	1-1	4,5 (0)	104,109(2)	-	*mut8* *mut2*
B	+	1-1	1,2 (0)	56,81 (0)	-	*mut2*
7A	+	1-1	0,0 (0)	7,9 (0)	+	+ +
B	-	1-7	175,188(59)	106,125(1)	-	*mut8* *mut2*
C	+	1-1	5,4 (0)	22,18 (0)	+	*mut8* +
D	-	1-7	116,100(7)	107,71 (14)	-	+ *mut2*
8A	-	1-7	157,167(59)	13,15 (0)	+	*mut8* +
B	+	1-1	1,1 (0)	73,92 (0)	-	+ *mut2*
C	+	1-1	0,0 (0)	13,14 (0)	+	+ +
9A	+	1-1	3,3 (0)	20,19 (0)	+	*mut8* +
B	+	1-1	0,4 (0)	259,234(202)*	-	+ *mut2*
C	-	1-7	111,120(70)	60,73 (0)	-	+ *mut2*
D	-	1-7	97,160(143)	37,19 (0)	+	*mut8* +
10A	-	1-7	67,90 (7)	83,79 (1)	-	+ *mut2*
B	+	1-1	10,8 (0)	372,367(282)*	-	*mut8* *mut2*
C	+	1-1	0,0 (0)	13,12 (0)	+	+ +
D	-	1-7	273,298(117)	13,22 (0)	+	*mut8* +

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (Inthese cases, unparenthesized numbers include pre-existing prototrophs.)

TABLE 85a: Summary of haploid Lassie scores from crosses RO82 and RO102

his1-7 reversion	Mutator(s)					
	MUT$^+$	*mut2*	*mut7*	*mut8*	*mut2mut7*	*mut2mut8*
Mean	9.7	93	45	157	96†	165†
S.E.	1.5	5.0	6.0	15	11	11
lys1-1 reversion						
Mean	11.4	82	43	20	115	97
S.E.	1.8	4	4.8	1.3	9.3	5.7

b: Mutation rates (M) in terms of (unreverted) cells per Lassie Plate of strains from crosses RO82 and RO102

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug * x 10^{-4}	M_{lys} x 10^8	Histidine Lassie score*	Cells/ plug * x 10^{-4}	M_{his} x 10^8
mut8	82-1A	1-7	99 (4)	178	23.3	97	149§	27.3
	102-7D	"	90	126	29.9	JP	117	
	82-8D	1-1	85 (1)	164	21.7	1	119	0.3
	102-6B	"	92 (8)	141§	27.3	2	105§	0.8
mut7	82-8C	1-7	46	86§	22.3	38	107	14.9
	-9C	1-1	44 (17)	147	12.5	0	127	<0.3
mut8	102-5A	1-7	38	144	11.1	72	120	25.1
	-7C	1-1	18 (5)	147	5.1	5	136	1.5
mut2, mut7	82-6B	1-7	JP (8)	121		111	117§	39.7
mut2, mut8	102-7B	1-7	JP	297		228	164§	58.2
	-6A	1-1	84 (8)	177§	19.9	4	146§	1.1
MUT$^+$	82-1D	1-7	11	208	2.2	19	190	4.2
	102-5B	1-7	9	189	2.0	28	106	11.1

* Average of two determinations
§ Average of four determinations
Numbers in parentheses are the number of red (locus) revertants/plate
† Only two spores tested

lysine reversion. The *lys1-1* mutation rates appear to be less than additive in *mut2 mut8* strains. It would be unwise to reject the null hypothesis of additivity prior to the testing of several *mut2 mut8* and *mut2 mut7* strains with the 1000-compartment fluctuation test, for either *his1-7* or *lys1-1* reversion.

c. *mut3* (Tables 86, 87 and 88)

Most *mut3* strains confer roughly five-fold enhancements of *lys1-1* and *his1-7* reversion rates. Suppressor revertants account for most of the increase. Nasim and Brychcy (1979) determined that this locus was also sensitive to MMS. Hence *mut3* is identified with segregating non-*ts*, MMS-sensitive spore clones. Note that *mut2*, *mut3*, *mut4* and *mut7* confer MMS-sensitivity. Therefore, the presence of the non-*mut7* mutator in double mutator mutant strains (from tetratype tetrads) had to be confirmed by complementation tests (Appendix, Table A2). This was also true of crosses where no markers sensitive to MMS, γ -irradiation or 36°C incubation were segregating.

The *mut3* locus may be linked to *mut7* (4P:6T:0N), but the numbers of tetrads scored was too low to be certain. It is unlikely that close linkage exists between *mut3* and *mut8* (3P:5T:1N). Again *his1-1* reversion is not unexpectly altered in *mut3*-bearing strains.

Additive enhancement of mutation rate cannot be excluded for either *mut7 mut3* or for *mut8 mut3*, based on differences and variability in frequencies and rates of *his1-7* or *lys1-1* reversion shown in Tables 88a and b.

TABLE 86: Phenotypes of spores recovered from cross R083 ($\frac{mut7}{+}$ $\frac{+}{mut3}$)

Strain R083-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival at MMS	Designated Mutator(s)	
1A	+	1-1	3 (0)	82 (0)	+	-	+	*mut3*
B	-	1-7	137 (16)	95 (123)	-	-	*mut7*	*mut3*
C	-	1-7	71 (0)	54 (0)	-	-	*mut7*	+
D	+	1-1	(0) (0)	14 (1)	+	+	+	+
2A	+	1-1	1 (0)	76 (3)	+	-	+	*mut3*
B	-	1-7	177 (191)*	86 (24)	-	-	+	*mut3*
C	-	1-7	50 (5)	35 (0)	-	-	*mut7*	+
D	+	1-1	1 (0)	35 (7)	-	-	*mut7*	+
3A	-	1-7	72 (8)	71 (1)	+	-	+	*mut3*
B	-	1-7	33 (12)	53 (0)	-	-	*mut7*	+
C	+	1-1	0 (0)	39 (0)	-	-	*mut7*	+
D	+	1-1	3 (0)	86 (6)	+	-	+	*mut3*
4A	-	1-7	11 (1)	14 (0)	+	+	+	+
B	-	1-1	3 (0)	228 (239)*	+	-	+	*mut3*
C	+	1-1	1 (0)	109 (1)	-	-	*mut7*	*mut3*
D	+	1-7	66 (0)	38 (0)	-	-	*mut7*	+
5A	-	1-7	64 (4)	30 (31)	-	-	*mut7*	+
B	+	1-1	0 (0)	11 (0)	+	+	+	+
C	-	1-7	59 (41)	76 (5)	+	-	+	*mut3*
6A	-	1-7	105 (89)	158 (2)	-	-	*mut7*	*mut3*
B	-	1-7	33 (5)	46 (0)	-	-	*mut7*	+
C	+	1-1	5 (0)	152 (2)	+	-	+	*mut3*
D	+	1-1	1 (0)	12 (0)	+	+	+	+
7A	+	1-1	2 (0)	91 (0)	+	-	+	*mut3*
B	-	1-7	79 (7)	81 (0)	+	-	+	*mut3*
C	-	1-7	55 (6)	48 (5)	-	-	*mut7*	+
D	+	1-1	0 (0)	43 (1)	-	-	*mut7*	+
8A	+	1-1	0 (0)	28 (0)	-	-	*mut7*	+
B	-	1-7	65 (8)	35 (29)	-	-	*mut7*	+
C	-	1-7	119 (6)	86 (0)	+	-	+	*mut3*
D	+	1-1	1 (0)	134 (3)	+	-	+	*mut3*
9A	-	1-7	9 (3)	10 (0)	+	+	+	+
B	-	1-7	130 (11)	106 (4)	-	-	*mut7*	*mut3*
C	+	1-1	2 (0)	80 (0)	+	-	+	*mut3*
D	+	1-1	2 (0)	30 (0)	-	-	*mut7*	+
10A	-	1-7	135 (2)	143 (4)	-	-	*mut7*	*mut3*
B	+	1-1	0 (0)	52 (0)	-	-	*mut7*	+
C	+	1-1	2 (0)	10 (0)	+	+	+	+
D	-	1-7	92 (0)	59 (0)	+	-	+	*mut3*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs

TABLE 87: Phenotypes of spores recovered from cross RO103($\frac{mut8}{+}$ $\frac{+}{mut3}$)

Strain RO103-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth on MMS	Designated mutator(s)
1A	+	1-1	5,5 (0)	72,64 (1)	-	*mut8 mut3*
B	-	1-7	307,256(95)	48,44 (23)	-	*mut8 mut3*
C	+	1-1	0,0 (0)	9,11 (0)	+	+ +
D	-	1-7	15,20 (0)	8,16 (0)	+	+ +
2A	-	1-7	222,193(66)	12,21 (0)	+	*mut8* +
B	+	1-1	1,5 (0)	80,67 (2)	-	*mut8 mut3*
C	-	1-7	10,11 (12)	12,10 (0)	+	+ +
D	+	1-1	2,1 (0)	68,91 (0)	-	+ *mut3*
3A	+	1-1	0,1 (0)	15,14 (0)	+	+ +
B	-	1-7	78,65 (60)	66,80 (1)	-	*mut3*
4A	-	1-7	181,181(141)	154,118(138)*	+	*mut8* +
B	-	1-7	118,97 (0)	68,82 (0)	-	+ *mut3*
C	+	1-1	0,2 (1)	55,60 (0)	-	+ *mut3*
D	+	1-1	2,5 (0)	15,11 (0)	+	*mut8* +
5A	-	1-7	12,18 (0)	9,12 (1)	+	+ +
B	+	1-1	2,6 (0)	85,64 (3)	-	*mut8 mut3*
C	+	1-1	4,4 (0)	14,19 (0)	+	*mut8* +
D	-	1-7	85,68 (2)	58,70 (0)	-	+ *mut3*
6A	+	1-1	1,1 (0)	7,9 (0)	+	+ +
B	-	1-7	55,34 (44)	45,85 (0)	-	+ *mut3*
C	-	1-1	6,5 (0)	95,52 (4)	-	*mut8 mut3*
D	+	1-7	*96,81* (57)	20,16 (0)	+	*mut8* +
7A	-	1-7	70,54 (4)	67,72 (0)	-	+ *mut3*
B	-	1-7	95,111(13)	69,65 (0)	-	+ *mut3*
C	+	1-1	2,1 (0)	15,12 (0)	+	*mut8* +
D	+	1-1	2,4 (0)	24,18 (0)	+	*mut8* +
8A	+	1-1	5,5 (0)	9,9 (0)	+	*mut8* +
B	-	1-7	34,47 (9)	57,79 (1)	-	+ *mut3*
C	+	1-1	7,5 (0)	69,91 (3)	-	*mut8 mut3*
D	-	1-7	12,9 (0)	14,11 (0)	+	+ +
9A	-	1-7	101,90 (104)	10,20 (0)	+	*mut8* +
B	-	1-7	59,41 (12)	83,42 (40)	-	+ *mut3*
C	+	1-1	1,7 (0)	12,13 (0)	+	*mut8* +
D	+	1-1	2,1 (0)	60,97 (0)	-	+ *mut3*
10A	-	1-7	273,227(67)	73,85 (0)	-	*mut8 mut3*
B	+	1-1	0,0 (0)	13,11 (0)	+	+ +
C	-	1-7	121,184(41)	24,24 (0)	+	*mut8* +

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 88a: Summary of haploid Lassie scores from crosses RO83 and RO103

his1-7 reversion	Mutator(s)					
	MUT$^+$	*mut3*	*mut7*	*mut8*	*mut3mut7*	*mut3mut8*
Mean	12.7	73	55	145	127	266†
S.E.	1.2	6	5.3	17	8	17
lys1-1						
Mean	11.5	77	41	16	122	71
S.E.	0.2	3.8	2.7	2.3	12	4.2

† Data from only two spores

b: Mutation Rates (M) of Strains from Crosses RO83 and RO103 in Terms of (unreverted) Cells per Lassie Plate

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug * x 10^{-4}	M_{lys} x 10^8	Histidine Lassie scores*	Cells/ plug * x 10^{-4}	M_{his} x 10^8
mut3	83-10D	1-7	49 (1)	117§	18	69	128	23
	103-6B	"	JP	202		79	132	25
	83-4B	1-1	70	105	28	2	192	0.4
	103-4C	"	82 (4)	175	20	0	127	<0.3
mut7	83-4D	1-7	37	93	17	64	110	24
	-10B	1-1	33	100§	14	1	111	0.4
mut8	103-6D	1-7	17 (12)	111	6.4	127	132	40
	-4D	1-1	19 (13)	160§	5.0	6	154	1.6
mut3, mut7	83-10A	1-7	78 (12)	146	22	106	124	36
	-4C	1-1	90 (14)	99§	38	3	125§	1.0
mut3, mut8	103-1B	1-7	77	152	21	226	113	84
		1-1	72 (1)	121	25	3	117§	1.1
MUT$^+$	83-4A	1-7	12 (2)	108	4.7	12	112	4.5
	103-1D	"	8 (1)	143	2.3	17	149	4.8

* Average of two determinations
§ Average of four determinations
Numbers in parentheses are numbers of red revertants/plate.

TABLE 89: Phenotypes of spores recovered from cross R084 ($\frac{mut7}{+}$ $\frac{+}{mut4}$)

Strain R084-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	on MMS	Designated Mutator(s)	
1A	+	1-1	4 (0)	73 (68)*	-	-	*mut7*	*mut4*
B	-	1-7	40 (4)	154 (182)*	+	-	+	*mut4*
C	+	1-1	0 (0)	14 (0)	-	-	*mut7*	+
D	-	1-7	9 (0)	18 (0)	+	+	+	+
2A	+	1-1	3 (0)	86 (26)	-	-	*mut7*	*mut4*
B	-	1-7	13 (0)	15 (0)	+	+	+	+
C	-	1-7	79 (8)	69 (3)	+	-	+	*mut4*
D	+	1-1	1 (0)	36 (4)	-	-	*mut7*	+
3A	+	1-1	2 (1)	69 (0)	+	-	+	*mut4*
B	+	1-7	32 (11)	53 (3)	-	-	*mut7*	+
C	-	1-1	0 (0)	10 (0)	+	+	+	+
D	-	1-7	99 (13)	69 (1)	-	-	*mut7*	*mut4*
4A	-	1-7	20 (1)	26 (0)	-	-	*mut7*	+
B	+	1-1	1 (0)	82 (4)	+	-	+	*mut4*
C	+	1-1	2 (0)	59 (12)	+	-	+	*mut4*
D	-	1-7	70 (4)	42 (0)	-	-	*mut7*	+
5A	+	1-1	1 (0)	56 (0)	+	-	+	*mut4*
B	-	1-7	51 (8)	79 (60)*	-	-	*mut7*	+
C	+	1-1	1 (0)	77 (1)	-	-	*mut7*	*mut4*
D	-	1-7	3 (3)	19 (1)	+	+	+	+
6A	+	1-1	0 (0)	33 (0)	-	-	*mut7*	+
B	+	1-1	0 (0)	53 (0)	+	-	+	*mut4*
C	-	1-7	27 (0)	10 (0)	-	-	*mut7*	+
D	-	1-7	61 (6)	104 (0)	+	-	+	*mut4*
7A	+	1-1	1 (0)	46 (0)	-	-	*mut7*	+
B	-	1-7	68 (5)	35 (0)	-	-	*mut7*	*mut4*
C	-	1-7	57 (4)	69 (87)	+	-	+	*mut4*
D	+	1-1	1 (0)	19 (0)	+	+	+	+
8A	+	1-1	0 (0)	38 (79)	-	-	*mut7*	*mut4*
B	+	1-1	0 (0)	10 (3)	+	+	+	+
C	-	1-7	34 (5)	23 (0)	-	-	*mut7*	+
D	+	1-7	38 (3)	40 (14)	+	-	+	*mut4*
9A	-	1-7	35 (0)	24 (9)	-	-	*mut7*	+
B	+	1-1	0 (0)	31 (12)	+	-	+	*mut4*
C	+	1-1	2 (0)	43 (0)	-	-	*mut7*	*mut4*
D	-	1-7	9 (0)	14 (0)	+	+	+	+
10A	-	1-7	8 (0)	7 (0)	+	+	+	+
B	+	1-1	2 (0)	57 (0)	-	-	*mut7*	*mut4*
C	+	1-1	0 (0)	7 (0)	+	+	+	+
D	-	1-7	65 (1)	72 (1)	-	-	*mut7*	*mut4*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs

TABLE 90: Phenotypes of spores recovered from cross RO104($\frac{mut8}{+}$ $\frac{+}{mut4}$)

Strain RO104-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth on MMS	Designated mutator(s)
1A	+	1-1	1,2 (0)	71,59 (0)	-	+ *mut4*
B	-	1-7	138,130(109)	27,16 (0)	+	*mut8* +
C	-	1-7	7,12 (1)	9,12 (0)	+	+ +
D	+	1-1	1,5 (1)	75,86 (6)	-	*mut8* *mut4*
2A	-	1-7	8,10 (0)	14,16 (1)	+	+ +
B	+	1-1	2,4 (1)	20,24 (7)	+	*mut8* +
C	-	1-7	76,98 (11)	55,59 (2)	-	*mut4*
3A	+	1-1	1,1 (0)	56,77 (4)	-	*mut4*
B	+	1-1	4,5 (0)	110,107(0)	-	+ *mut4*
C	-	1-7	86,168(165)	22,16 (0)	+	*mut8* +
4A	+	1-1	5,6 (0)	91,80 (3)	-	*mut8* *mut4*
B	+	1-1	0,0 (0)	10,9 (0)	+	+ +
C	-	1-7	167 (85)	20,22 (0)	+	*mut8* +
D	-	1-7	76,88 (6)	84,70 (0)	-	+ *mut4*
5A	+	1-1	3,2 (0)	31,12 (19)	+	*mut8* +
B	-	1-7	182,165(94)	20,20 (0)	+	*mut8* +
C	-	1-7	97,103(2)	74,42(52)	-	+ *mut4*
D	+	1-1	12,5 (0)	59,94 (0)	-	+ *mut4*
6A	+	1-1	6,4 (0)	22,20 (0)	+	*mut8* +
B	+	1-1	5,11 (0)	65,110(0)	-	*mut8* *mut4*
C	-	1-7	16,22 (0)	7,12 (0)	+	+ +
D	-	1-7	80,87 (0)	59,100(0)	-	+ *mut4*
7A	+	1-1	2,5 (0)	81,95 (0)	-	*mut8* *mut4*
B	-	1-7	201,174(96)	74,73 (0)	-	*mut8* *mut4*
C	-	1-7	3,10 (6)	9,5 (0)	+	+ +
D	+	1-1	0,0 (0)	4,4 (0)	+	+ +
8A	-	1-7	84,81 (3)	39,73 (1)	-	+ *mut4*
B	+	1-1	2,4 (0)	93,119(2)	-	*mut8* *mut4*
C	+	1-1	0,0 (0)	9,15 (0)	+	+ +
D	-	1-7	140,166(55)	8,22 (0)	+	*mut8* +
9A	+	1-1	2,3 (0)	79,71 (0)	-	+ *mut4*
B	-	1-7	158,161(41)	8,28 (0)	+	*mut8* +
C	+	1-1	0,1 (0)	79,107(0)	-	+ *mut4*
D	-	1-7	171,184(17)	13,27 (0)	+	*mut8* +
10A	+	1-1	6,4 (0)	26,22 (0)	+	*mut8* +
B	+	1-1	0,0 0)	38,91 (3)	-	+ *mut4*
C	-	1-7	184 (93)	19,21 (0)	+	*mut8* +
D	-	1-7	64 (4)	65,90 (31)	-	+ *mut4*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.

TABLE 91a: Summary of haploid Lassie scores from crosses RO84 and RO104

his1-7 reversion	Mutator(s)					
	MUT$^+$	*mut4*	*mut7*	*mut8*	*mut4mut7*	*mut4mut8*
Mean	10.6	76	38	157	77†	188†
S.E.	1.4	4.8	6.4	8	11	14
lys1-1 reversion						
Mean	11.2	71	31	20	58	87
S.E.	1.0	3.9	4.4	1.2	7.8	5.0

† Fewer than four spores tested

b: Mutation rates (M) of strains from crosses RO84 and RO104 in terms of (unreverted) cells per Lassie plate

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug* x 10^{-4}	M_{lys} x 10^8	Histidine Lassie score*	Cells/ plug* x 10^{-4}	M_{his} x 10^8
mut4	84-1B	1-7	56	168	14	91	131	29
	84-3A	1-1	66 (1)	121	23	1	142	0.3
	104-1A	"	JP (1)	265		1	129	0.3
mut7	84-3B	1-7	42	128	14	64	159	17
	-1C	1-1	42	142	12	1	123	0.3
mut8	104-1B	1-7	18	160	4.7	168	113	62
	-5A	1-1	31 (17)	169	7.7	5	104	2.0
mut4, mut7	84-3D	1-7	118	144	34	97	133	31
	-1A	1-1	JP (1)	170		0	84	<0.5
mut4, mut8	104-7B	1-7	93	232	17	102	72	59
MUT$^+$	84-3C	1-1	7 (1)	139	2.1	1	149	0.3

* Average of two determinations
§ Average of four determinations
Numbers in parentheses are numbers of red (locus) revertants/plate

d. *mut4* (Tables 89, 90, and 91)

The *mut4* locus confers an approximate four-fold increase in haploid reversion for both *his1-7* and *lys1-1*, and Nasim and Brychcy (1979) have shown that the locus is MMS sensitive. The MMS-sensitivity was again exploited to detect the *mut4* locus.

Both *mut7* and *mut8* appear to assort independently of *mut4* (2P:7T:1N and 3P:5T:1N, respectively). The *mut 4* locus does not confer enhanced *his1-1* reversion.

The *mut4 mut7* and *mut4 mut8* strains appear to be less than additively enhanced for *his1-7* reversion (Tables 91a and 91b). These strains were additively enhanced for *lys1-1* reversion.

e. *mut5* and *rad51-1* (Tables 92 to 108)

The *mut5-1* allele confers a five to ten-fold increase in spontaneous mutation at both *his1-7* and *lys1-1*. It has been shown to confer U.V., γ and MMS-sensitivities (Quah, unpublished, Nasim and Brychcy 1979). It is also responsible for reduced UV-induced intragenic recombination at *his1*, and is allelic with *rad51* (Morrison, 1978). For this study, the γ -radiation sensitivity was used to detect *mut5* strains.

The *mut5* allele is not closely linked to *mut7* (7P:9T:0P suggested linkage from Table 92, but these ratios indicating linkage were not found in subsequent crosses, for example, Table 96) nor to *mut8* (3P:6T:0N). The *mut5* allele did not enhance *his1-1* reversion significantly (Tables 92 and 93).

The *lys1-1* and *his1-7* reversion scores appeared to be less than additively enhanced for *mut8 mut5* strains according to both Tables 94a and 94b, compared to strains bearing either single mutant. Viable *mut7 mut5* strains were not detected in the RO85 cross (Table 92). No spore clones with both γ -sensitive and *ts* phenotypes were found in thirty-three more tetrads dissected from this cross. Two crosses, of daughter strains RO85-2C (*mut7*)/RO85-2B (*mut5*), and RO85-17D (*mut7*)/ RO85-17B (*mut5*) failed to uncover any viable *mut7 mut5* strains in a total of 26 tetrads tested. It appeared that *mut7* conferred inviability to *mut5* strains or vice-versa. A cross of the *mut7* strain (RO428-6B) to the *rad51-1* strain RO91-1C produced no viable *mut7 rad51-1* segregants in 16 tetrads. Hence this phenotype appeared to be a characteristic trait conferred by *rad51* alleles.

Table 95 indicates that the above premise was not always true. Four four-spored tetrads dissected from cross RO112 bore viable *mut7 mut5 mut8* spores. To ascertain that this viability of the strains bearing *mut7 mut5* was not due to the presence of *mut8*, a *mut7 mut8 mut5* strain (RO112-1B) was crossed to the *MUT*$^{+}$ *his1-7* strain RO255-3A. As may be seen from Table 96, approximately half of the viable *mut7 mut5* segregants did not contain *mut8* (8/14). Of inviable *mut7 mut5* spore clones (at least 11/25), roughly half (4/11) contained *mut8*. Hence, it is unlikely that the presence of *mut8* was responsible for the viability of the previously inviable combination. It is possible that *mut7 mut5* strains may be rescued by the presence of another segregating locus, based on the viability of about one half of the presumed *mut7 mut5* spores.

The RO113 cross also suggested that *mut7* was linked to *lys1-1* (9P:15T:0N). No other linkages were observed except that of *mut5* to *hom3* (11P:13T:0N), which produced a map distance comparable to those calculated by Morrison (1978). The observation was peculiar because two other crosses, RO250 and RO252 had yielded completely opposite results for *mut7* - *lys1* linkage (a total of OP:12T:6N, for both crosses). The only major difference between these crosses was that *mut5* was not segregating in the latter two. One explanation could therefore be that *mut5* caused a reduction in *mut7*-mediated recombination between distant markers on the same chromosome. A second cross, of the haploid RO112-1B (*mut7 mut8 mut5*) to the *MUT*$^+$ strain RO107-5C which bore the usual markers (strain RO118, Table 97) yielded a similar proportion of viable *mut7 mut5* spore clones (3/5).

Both crosses RO113 and RO118 had reduced numbers of revertants in many spore clones, even after 14 days' incubation, compared with cross RO112 (Tables 98a,b and c) or RO105 (Tables 93 and 94). In fact, the only *mut7 mut8 his1-7* strain segregating in cross RO118 (2A) showed a halving of the Lassie scores (Tables 97 and 102d) seen in the *mut7 mut8* data shown in Tables 98a and b. The *mut7 mut8 mut5* strain RO118-3C had a reduced lysine locus revertant frequency when compared with the usual *mut7 mut8*-mediated locus scores (see, for example, Table 9). This suggests that the reduction in total lassie score may be due to the elimination of the *lys1* locus category of enhanced reversion in *mut7 mut8* strains.

In further attempt to explore the possibility that an antimutator gene was causing the reduced mutation frequencies seen in

the above examples, the data shown in Table 99 were collected. The diploid strains bearing various doses of *mut7* and *mut8* were included for comparison. It is evident that the two *mut7* haploids RO428-6B and 6C have relatively enhanced mutation rates compared to other *mut7* strains (Table 9). The reduction in *mut7 mut8*-mediated reversion rates in spore clones from RO112, even compared to the rates for *mut7 mut8 mut5* strains is again apparent. Interestingly enough, *mut7 mut8 mut5* strains also have a reduced "*mut8* effect" (Tables 95,96 and 97).

All spore germinations subsequent to those of diploid RO94 (the RO100 and RO500 series) were performed at 20°C following the observation that viable *mut7 rad52* spore clones were recoverable at 15°C but not at 26°C (Tables 122 and 123). While spores from crosses of *mut7* to *mut5* were never repeated at this low temperature, it was considered necessary to repeat, at 20°C, the specific cross that had first shown the lethal interaction of *mut5* and *mut7* (RO428-6B/RO75-4D). The *mut7* revertant isolated from RO428-6B, *mut7-1-11*, was also crossed to the same *mut5* strain to ensure that the observed lethality was not occurring because of other genetic factors present in the parents of the original *mut7/mut5* cross. Ten tetrads each were sporulated, and then germinated at the following respective temperatures; 20°C/20°C, 26°C/26°C, 20°C/26°C, and 26°C/20°C for each of the two crosses. The results may be seen in Tables 100 to 107. No viable *mut7 mut5* spore clones were observed to segregate under any of the above conditions. The cross of *mut7-1-11* to *mut5* showed no patterns of spore lethality (9/80 inviable *mut5* spores, compared to 2/24 inviable

TABLE 92 Phenotypes of spores recovered from cross RO85 ($\frac{mut7}{+} \times \frac{+}{mut5}$)

Strain RO85-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising on limiting histidine	Prototrophs arising on limiting lysine	Survival at after 36°C	Survival at after γ	Designated Mutator(s)	
1A	+	1-1	2 (0)	74 (13)	+	-	+	*mut5*
B	+	1-1	1 (0)	8 (1)	+	+	+	+
C	-	1-7	53 (12)	30 (0)	-	+	*mut7*	+
2A	+	1-1	1 (0)	27 (0)	+	+	+	+
B	-	1-7	64 (40)	51 (3)	+	-	+	*mut5*
C	+	1-1	0 (0)	45 (0)	-	+	*mut7*	+
3A	-	1-7	83 (10)	48 (9)	+	-	+	*mut5*
B	-	1-7	44 (14)	61 (0)	+	-	+	*mut5*
4A	-	1-7	64 (1)	52 (3)	+	-	+	*mut5*
B	+	1-1	0 (0)	39 (15)	-	+	*mut7*	+
C	-	1-7	41 (4)	66 (0)	+	-	+	*mut5*
D	+	1-1	0 (0)	31 (0)	-	+	*mut7*	+
5A	-	1-7	42 (1)	22 (0)	-	+	*mut7*	+
B	-	1-7	68 (6)	61 (4)	+	-	+	*mut5*
C	+	1-1	1 (0)	444 (522)*	+	-	+	*mut5*
D	+	1-1	4 (0)	42 (0)	-	+	*mut7*	+
6A	+	1-1	2 (0)	52 (1)	-	+	*mut7*	+
B	-	1-7	48 (23)	56 (1)	+	-	+	*mut5*
C	+	1-1	0 (1)*	7 (0)	+	+	+	+
7A	+	1-1	0 (0)	17 (0)	+	+	+	+
B	-	1-7	60 (20)	46 (15)	+	-	+	*mut5*
C	+	1-1	0 (0)	33 (0)	-	+	*mut7*	+
8A	-	1-7	26 (1)	15 (0)	-	+	*mut7*	+
B	-	1-7	66 (4)	56 (9)	+	-	+	*mut5*
C	+	1-1	2 (0)	52 (1)	+	-	+	*mut5*
9A	+	1-1	0 (0)	30 (0)	-	+	*mut7*	+
B	-	1-7	52 (1)	66 (0)	+	-	+	*mut5*
C	+	1-1	1 (0)	39 (1)	-	+	*mut7*	+
10A	-	1-7	32 (80)	66 (5)	+	-	+	*mut5*
B	+	1-1	0 (0)	55 (0)	-	+	*mut7*	+
C	+	1-1	0 (0)	15 (0)	+	+	+	+
11A	+	1-1	0 (0)	8 (0)	+	+	+	+
B	-	1-7	47 (6)	56 (24)	+	-	+	*mut5*
12A	+	1-1	1 (0)	40 (2)	-	+	*mut7*	+
B	+	1-1	1 (0)	12 (0)	+	+	+	+
C	-	1-7	83 (4)	77 (19)	+	-	+	*mut5*
13A	+	1-1	0 (0)	8 (0)	+	+	+	+
B	-	1-7	373 (340)*	188 (174)*	+	-	+	*mut5*
C	+	1-1	0 (0)	57 (1)	-	+	*mut7*	+

(cont'd)

(cont'd)

Strain RO85-	Segregating alleles at *hom3*	*his1*	Prototrophs arising on limiting histidine	lysine	Survival at after 36°C	γ	Designated Mutator(s)	
14A	-	1-7	90 (1)	51 (3)	+	-	+	*mut5*
B	+	1-1	2 (0)	39 (0)	-	+	*mut7*	+
C	+	1-1	0 (0)	35 (0)	-	+	*mut7*	+
D	-	1-7	57 (21)	85 (2)	+	-	+	*mut5*
15A	-	1-7	169 (143)*	54 (2)	+	-	+	*mut5*
B	-	1-7	13 (0)	14 (0)	+	+	+	+
C	+	1-1	1 (0)	31 (2)	-	+	*mut7*	+
16A	+	1-1	2 (0)	14 (0)	+	+	+	+
17A	+	1-1	0 (0)	78 (0)	+	-	+	*mut5*
B	-	1-7	56 (56)	72 (7)	+	-	+	*mut5*
C	+	1-1	2 (0)	47 (9)	-	+	*mut7*	+
D	-	1-7	44 (3)	39 (0)	-	+	*mut7*	+

* Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

** 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs

TABLE 93: Phenotypes of spores recovered from cross RO105 ($\frac{mut8}{+}$ $\frac{+}{mut5}$)

Strain RO105-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Growth after γ	Designated mutator(s)
1A	-	1-7	10,14 (0)	13,14 (0)	+	+ +
B	-	1-7	154,153(53)	20,16 (0)	+	*mut8* +
C	+	1-1	2,7 (0)	68,57 (0)	-	*mut8 mut5*
D	+	1-1	3,1 (0)	72,68 (6)	-	+ *mut5*
2A	-	1-7	178,137(42)	69,67 (0)	-	*mut8 mut5*
B	+	1-1	0,0 (0)	13,9 (0)	+	+ +
C	-	1-7	153,134(14)	21,16 (0)	+	*mut8* +
D	+	1-1	3,3 (0)	48,65 (47)	-	+ *mut5*
3A	-	1-7	252,244(188)*	13,14 (0)	+	*mut8* +
B	-	1-7	64,71 (1)	61,68 (0)	-	+ *mut5*
C	+	1-1	1,0 (0)	66,60 (0)	-	+ *mut5*
D	+	1-1	2,6 (0)	17,15 (0)	+	*mut8* +
4A	-	1-7	167,160(13)	24,22 (0)	+	*mut8* +
B	+	1-1	3,3 (0)	73,61 (0)	-	*mut5*
5A	-	1-7	47,70 (1)	79,64 (1)	-	+ *mut5*
B	+	1-1	4,5 (0)	18,17 (1)	+	*mut8* +
C	+	1-1	4,3 (0)	61,69 (0)	-	*mut8 mut5*
D	-	1-7	10,8 (0)	10,6 (0)	+	+ +
6A	+	1-1	2,4 (0)	70,73 (0)	-	*mut8 mut5*
B	+	1-1	2,2 (0)	71,56 (11)	-	+ *mut5*
C	-	1-7	9,14 (0)	9,12 (0)	+	+ +
D	-	1-7	120,140(14)	18,11 (0)	+	*mut8* +
7A	+	1-1	0,1 (0)	68,91 (0)	-	+ *mut5*
B	+	1-1	1,1 (0)	69,65 (0)	-	+ *mut5*
C	-	1-7	103,91 (59)	17,15 (0)	+	*mut8* +
D	-	1-7	186,189(4)	18,19 (0)	+	*mut8* +
8A	-	1-7	11,7 (0)	16,7 (0)	+	+ +
B	-	1-7	152,147(13)	14,11 (0)	+	*mut8* +
C	+	1-1	1,1 (0)	60,67 (19)	-	+ *mut5*
D	+	1-1	3,4 (0)	77,63 (29)	-	*mut8 mut5*
9A	+	1-1	0,1 (0)	56,72 (10)	-	+ *mut5*
B	+	1-1	1,1 (0)	69,62 (3)	-	+ *mut5*
C	-	1-7	107,137(42)	15,16 (0)	+	*mut8* +
10A	+	1-1	2,1 (0)	67,66 (0)	-	+ *mut5*
B	+	1-1	3,3 (0)	13,16 (0)	+	*mut8* +
C	-	1-7	16,17 (0)	20,8 (0)	+	+ +
D	-	1-7	180,155(48)	61,74 (0)	-	*mut8 mut5*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 94a: Summary of haploid Lassie scores from crosses RO85 and RO105

his1-7 reversion	Mutator(s) *MUT*$^+$	*mut5*	*mut7*	*mut8*	*mut5mut8*
Mean	11.9	60	41	143	163
S.E.	1.0	3.4	5.7	7	11
lys1-1 reversion					
Mean	12.1	64	38	16.5	67
	1.8	1.5	2.5	0.7	1.8

b: Mutation rates (M) of strains from cross RO105 in terms of unreverted cells per Lassie test plate

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug * x 10^{-4}	M_{lys} x 10^{8}	Histidine Lassie score*	Cells/ plug * x 10^{-4}	M_{his} x 10^{8}
mut5	105-3B	1-7	84	145§	24	68	92§	31
	-1D	1-1	54	93§	24	1	131§	0.3
mut8	105-1B	1-7	16 (1)	135	5.0	180	154	49
	-3D	1-1	17 (8)	124	5.7	5	133	1.6
			24 (2)	197	5.1	3	137	0.9
mut5, mut8	105-2A	1-7	88	159§	23	156	111	59
	-1C	1-1	48(10)	133§	15	3	126§	1.0
MUT$^+$	105-1A	1-7	16 (1)	130	5.2	28	180	6.5
	-2B	1-1	9 (2)	144	2.6	1	155	0.3

* Average of two determinations
§ Average of four determinations
Numbers in parentheses are numbers of red (lysine locus) revertants

TABLE 95: Phenotypes of spores from cross RO112($\frac{mut7}{+}$ $\frac{mut8}{mut8}$ $\frac{+}{mut5}$)

Strain RO112-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	after γ	Designated mutator(s)
1A	All are		151,138(138)	82,103(4)	+	-	+ 8 5
B	*hom3-10*,		345,347(33)	14,22 (2)	-	-	7 8 5
C		*his1-7*.	107,120(107)	14,20 (0)	+	+	+ 8 +
D			643,425(268)	58,66 (2)	-	+	7 8 +
2A			140,140(115)	67,67 (72)	+	-	+ 8 5
B			611,656(432)	93,83 (14)	-	+	7 8 +
C			197,178(214)	14,16 (0)	+	+	+ 8 +
D			730,779(31)	57,53 (1)	-	-	7 8 5
3A			389,414(41)	58,51 (2)	-	-	7 8 5
B			1623,1501(1592)*	126,100(0)	-	+	7 8 +
C			109,94 (207)	99,93 (0)	+	-	+ 8 5
D			68,99 (107)	25,24 (1)	+	+	+ 8 +
4A			160,182(199)	121,75 (31)	+	-	+ 8 5
B			528,595(641)	129,107(11)	-	+	7 8 +
C$^{\rho}$			59,46 (7)	33,31 (6)	+	-	+ 8 5
D$^{\rho}$			716,575(39)	71,56 (1)	-	+	7 8 +
5A			297,323(25)	59,38 (2)	-	-	7 8 5
B			143,96 (231)	78,102(5)	+	-	+ 8 5

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

ρ These spores failed to grow when replica-plated to YG medium.

TABLE 9α Phenotypes of spores recovered from cross RO113 (*mut7*/+ *mut8*/+ *mut5*/+ *hom3*/+ *his1-7*/*his1-7* *lys1*/+ *ade2*/+ *trp5*/+ *arg4*/+)

Strain RO113-	Segregating alleles at: *lys1*	*hom3*	*ade2*	*arg4*	*trp5*	Prototrophs arising** on limiting histidine	Survival at 36°C	Survival after γ	Designated mutator(s)
1A	-	+	+	+	+	15,12 (1)	-	-	*7* + *5*
B	+	-	-	-	-	80,74 (112)	+	+	+ *8* +
C	-	+	+	+	-	1362,1275(1315)*	-	+	*7* +
D†									
2A	-	+	-	+	-	94,89 (97)	+	-	+ *8* *5*
B	-	-	+	-	-	22,11 (0)	-	-	*7* + *5*
C	+	-	-	-	+	34,23 (8)	-	+	*7* + +
D	+	+	+	+	+	119,109 (29)	+	+	+ *8* +
3A	-	+	+	+	-	535,583(140)	-	+	*7* *8* +
B	-	-	+	-	+	32,19 (9)	+	-	+ + *5*
C	+	+	-	-	-	7,13 (0)	-	+	*7* + +
D†									
4A	-	+	-	+	-	551,532 (85)	-	+	*7* *8* +
B	-	-	+	+	-	18,29 (39)	-	+	*7* + +
5A	-	-	-	-	-	16,13 (0)	-	-	*7* *5*
B	-	+	+	+	+	26,21 (0)	-	-	*7* + *5*
C	+	-	+	+	+	68,84 (16)	+	+	+ *8* +
D	+	+	-	-	-	32,45 (8)	+	+	+ *8* +
6A	+	+	-	+	+	46,46 (12)	+	-	+ + *5*
B	-	-	-	+	+	537,539(120)	+/-	-	*7* *8* +
C	+	+	+	-	-	58,65 (8)	+	+	+ *8* +
7A	-	+	+	-	-	13,19 (6)	+	+	+ + +
B	+	-	-	-	+	31,49 (60)	+	+	+ *8* +
8A	-	-	+	+	+	68,79 (17)	+	+	+ *8* +
B	-	+	-	+	-	20,19 (22)	-	+	*7* + +
C	+	-	+	-	-	111,117 (32)	+	-	+ *8* *5*
9A	+	+	+	+	+	131,84 (3)	+	+	+ *8* +
B	-	+	+	-	-	718,642 (69)	-	+	*7* *8* +
C	+	-	-	-	-	28,16 (0)	+	-	+ + *5*
D	-	-	-	+	+	18,21 (1)	-	-	*7* + *5*
10A	-	-	-	-	+	12,15 (0)	+	+	+ + +
B	-	+	-	+	-	12,16 (1)	-	+	*7* + +
C	+	+	+	+	-	85,116 (1)	+	-	+ *8* *5*
D	+	-	+	-	+	432,351 (20)	-	-	*7* *8* *5*
11A°	-	-	-	-	+	11,16 (0)	-	-	*7* + *5*
B	+	+	+	+	-	80,68 (2)	+	+	+ *8* +
C	+	+	+	-	+	34,31 (0)	-	+	*7* + +
D	+	-	-	+	-	110,131 (9)	+	-	+ *8* *5*

TABLE 96: (continued)

Strain RO113-	Segregating alleles at: *lys1*	*hom3*	*ade2*	*arg4*	*trp5*	Prototrophs arising** on limiting histidine	Survival at 36°C	after γ	Designated mutator(s)
12A	+	-	+	+	+	80,92 (1)	+	+	+ 8 +
B	+	+	-	-	-	47,72 (1)	+	-	+ + 5
C	-	+	-	+	+	13,3 (2)	-	+	7 + +
D	-	-	+	-	-	290,296(34)	-	-	7 8 5
13A	+	+	-	-	-	45,69 (94)	+	+	+ 8 +
B	-	-	+	+	+	53,44 (4)	+	-	+ + 5
C	-	-	-	-	-	10,13 (2)	-	-	7 + 5
D	+	+	+	+	+	376,350(39)	+	+	7 8 +
14A	+	-	+	-	-	31,29 (2)	+	-	+ + 5
B	+	+	-	-	-	76,116(105)	+	+	+ 8 +
C	-	+	+	+	+	657,622(81)	-	+	7 8 +
15A	-	-	+	+	-	93,109(11)	+	-	+ 8 5
B	+	+	-	-	+	18,13 (3)	+	+	+ + +
16A	-	+	+	-	-	30,18 (1)	-	+	7 + +
B	+	-	+	+	-	227,226(13)	-	-	7 8 5
C	+	+	-	+	+	109,130(69)	+	+	+ 8 +
D	-	-	-	-	+	38,27 (1)	+	-	+ + 5
17A	+	+	+	+	+	661,593(238)	-	+	7 8 +
18A	-	+	-	-	-	337,319(149)	-	+	7 8 +
B	+	+	+	+	+	94,81 (13)	+	+	+ 8 +
19A°	-	+	+	-	+	37,40 (34)	+	+	+ 8 +
B	+	+	+	+	-	3,4 (0)	+	+	+ + +
C†									
20A	+	-	-	-	-	7,4 (0)	+	+	+ + +
B	-	-	+	+	+	15,18 (3)	-	-	7 + 5
C	+	+	+	-	-	96,102(47)	+	+	+ 8 +
21A	+	+	-	+	-	18 (2)	-	+	7 + +
B	-	+	+	-	+	170,153(33)	+	+	+ 8 +
C°	-	-	-	-	+	241,177(8)	-	-	7 8 5
D	+	-	+	+	-	35,53 (0)	+	-	+ + 5
22A	+	-	+	-	-	329,280(95)	-	-	7 8 5
B	+	-	-	+	-	15,6 (2)	+	+	+ + +
C	-	+	+	-	+	100,83 (4)	+	-	+ 8 5
D	-	+	-	+	+	29,17 (0)	-	+	7 + +
23A	+	+	-	-	+	24,32 (2)	-	-	7 + 5
B	-	+	+	+	-	15,24 (0)	+/-	+	+ + +
C	-	-	-	+	-	519,389(61)	-	+	7 8 +
D	+	-	+	-	+	114,121(86)	+	-	+ 8 5
24A	-	-	-	-	-	57,65 (98)	+	-	+ 8 5
B	+	+	+	-	+	133,151(17)	+	+	+ 8 +
C	-	+	+	-	-	18,13 (0)	-	+	7 + +

TABLE 96: (continued)

Strain RO113-	Segregating alleles at: *lys1*	*hom3*	*ade2*	*arg4*	*trp5*	Prototrophs arising** on limiting histidine	Survival at 36°C	Survival after γ	Designated mutator(s)		
25A	+	-	-	+	+	26,32 (28)	+	-	+	+	5
B	+	+	+	+	-	590,763(71)	-	+	7	8	+
C	-	+	+	-	+	119,120(4)	+	-	+	8	5
26A	+	-	+	+	+	32,30 (0)	+	-	+	+	5
B	-	+	+	-	-	693,562(180)	-	+	7	8	+
C	+	+	-	+	+	7,10 (19)	+	+	+	+	+

** Numbers of prototrophs arising prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

ρ These spores failed to grow when replica-plated to YG medium.

† Semi-lethal, 'pinpoint' spore colonies were observed but were untransferable.

TABLE 97: Phenotypes of spores recovered from cross RO118 ($\frac{mut7}{+}$ $\frac{mut8}{+}$ $\frac{mut5}{+}$)

Strain RO118-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival after γ	Designated mutator(s)
1A	-	1-7	33,32 (2)	26,33 (0)	-	-	*7* + *5*
B	+	1-1	4,5 (0)	7,14 (0)	-	-	*7* *8* *5*
C	+	1-1	0,0 (0)	9,23 (0)	+	+	+ + +
D	-	1-7	219,245(13)	26,18 (0)	+	+	+ *8* +
2A†	-	1-7	294,256(6)	16,18 (0)	-	+	*7* *8* +
B	+	1-1	0,1 (0)	25,19 (0)	-	+	*7* + +
C	-	1-7	151,179(10)	56,79 (2)	+	-	+ *8* *5*
D	+	1-1	0,0 (0)	68,68 (2)	+	-	+ + *5*
3A	-	1-7	19,45 (0)	40,26 (0)	+	-	+ + *5*
B	+	1-1	0,0 (0)	13,14 (0)	-	+	*7* + +
C†	-	1-7	416,370(10)	6,11 (0)	-	-	*7* *8* *5*
D	+	1-1	4,3 (0)	16,13 (0)	+	+	+ *8* +
4A	-	1-7	123,13 (15)	14,19 (0)	+	+	+ *8* +
B	+	1-1	8,6 (1)	85,104(0)	+	-	+ *8* *5*
C	+	1-1	0,1 (0)	21,16 (0)	-	+	*7* + +
5A	-	1-7	151,115(28)	79,65 (5)	+	-	+ *8* *5*
Bρ	+	1-1	0,0 (0)	3,11 (0)	-	+	*7* + +
C	-	1-7	10,6 (22)	18,20 (0)	-	+	*7* + +
D	+	1-1	5,5 (0)	77,116(1)	+	-	+ *8* *5*
6A	+	1-1	0,1 (0)	26,23 (0)	+	-	+ + *5*
B	+	1-1	1,0 (0)	23,18 (0)	-	+	*7* + +
7A	+	1-1	19,22 (0)	67,70 (2)	-	+	*7* *8* +
B	-	1-7	7,4 (0)	14,12 (0)	+	+	+ + +
C	-	1-7	55,54 (1)	37,48 (0)	+	-	+ + *5*
D	+	1-1	2,0 (0)	4,0 (0)	-	-	*7* *8* *5*
8A	-	1-7	31,29 (1)	20,18 (23)	-	+	*7* + +
9A	+	1-1	1,0 (0)	28,15 (0)	-	+	*7* + +
B	+	1-1	24,13 (0)	92,106(1)	-	+	*7* *8* +
C	-	1-7	116,66 (3)	38,29 (5)	+	-	+ *8* *5*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† Spores are *ade*$^+$ or show high adenine reversion on replica plates.

ρ This spore failed to grow when replica-plated to YG medium.

TABLE 98a: Summary of haploid Lassie test scores from cross RO112

his1-7 reversion	Mutator(s) *mut8*	*mut7mut8*	*mut5mut8*	*mut5mut7mut8*
Mean	128	594	122	453
S.E.	21	32	12	68
lys1-1 reversion				
Mean	19	89	79	44
S.E.	2.1	8.5	8	6.2

b: Summary of haploid Lassie test scores from cross RO113

his1-7 reversion	Mutator(s) *MUT*+	*mut7*	*mut8*	*mut5*	*mut8mut5*	*mut7mut5*	*mut7mut8*	*mut5, mut7mut8*
Mean	11.6	19.8	86	37	102	17.8	567	285
S.E.	1.6	1.9	6	3	5	1.6	26	24

c: Summary of haploid Lassie test scores from cross RO118

his1-7 reversion	Mutator(s) *MUT*+	*mut7*	*mut8*	*mut5*	*mut8mut5*	*mut7mut5*	*mut7mut8*	*mut5, mut7mut8*
Mean	5.5	19	179	43	130	33	275	393
S.E.	1.5*	6.5	31	9	17	1*	19*	23*
lys1-1 reversion								
Mean	14.5	16.2	18	42	73	30	62	7
S.E.	3.1	1.6	2	6.4	8.6	4*	16	2.1

* Only one spore clone (two determinations) tested.

d: Mutation rates of strains from crosses RO113 and RO118 in terms of (unreverted)

Mutator	RO strain	Lysine Lassie score*	Cells/ plug* x 10^{-4}	M_{lys} x10^{8}	*his1* allele	Histidine Lassie score*	Cells/ plug* x 10^{4}	M_{his} x10^{8}
mut7	118-2B	18	118§	6.4	1-1	0	98	0.4
mut7mut8	118-2A	31	101§	12.9	1-7	424	151	118
mut7mut5	118-1A	32	101§	13.2	1-7	35	64	23
	113-1A	29	67§	18	1-7	10	59§	7.1
mut7mut8, mut5	118-3C	48(5)†	118§	17	1-7	377	97	162
	-1B	25	77§	14	1-1	7	44	6.7
	-7D	21	84	11	1-1	1	45	0.9

† Numbers of locus revertants are in parentheses.
* Two determinations per strain. § Four determinations per strain

TABLE 99 : Mutation rates of various strains (M) in terms of (unreverted) cells per lassie plate

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug $\times 10^{-4}$	M_{lys} $\times 10^{8}$	Histidine Lassie score*	Cells/ plug $\times 10^{-4}$	M_{his} $\times 10^{8}$
MUT^{+}	400-10C	1-7	9 (1)**	128*	2.9	12	123*	4.1
			17	695 †	2.0	10	90	4.7
	428-6C^{T}12	"	17 (2)	172*	4.1	17	124*	5.7
	107-5C	1-1	19	379 †	4.2	0	169	< 0.3
	428-6B^{T}11	"	14 (1)	114*	5.1	0	86*	< 0.5
mut7	428-6C	1-7	40 (12)	134*	12	46	112*	17
			38	294*†	11	49	117*	18
	-6B	1-1	44 (13)	133*	14	1	92*	0.5
			42	294*†	12	0	96*	< 0.4
mut8	108-1C	1-7	31	470*†	5.5	83	104*	33
mut8mut9	*89-6C*	"	72	403 †	15	79	90	37
mut8mut5	112-1A	1-7	103	178 †	48	151	70	90
mut8mut5mut7	B	"	14	186 †	6.3	345	31	466
mut8	C	"	14	246 †	4.7	107	110	41
mut8mut7	D	"	58	350 †	14	643	167	161
mut8mut5	-2A	1-7	67	299 †	19	140	100	59
mut8mut7	B	"	93	352 †	22	611	165	155
mut8	C	"	14	424 †	2.7	178	113	52
mut8mut5mut7	D	"	57	254 †	19	730	84	364
$\frac{mut7}{mut7}$	516	$\frac{1-7}{1-1}$	39	216*†	15	212	59*	150
$\frac{mut8\text{-}2mut9}{+ \quad +}$ $\frac{+}{mut7}$	508	$\frac{1-7}{1-1}$	19	251 †	6.3	80	40	84
$\frac{mut8\text{-}2mut9}{mut8\text{-}1 \quad +}$	514	$\frac{1-7}{1-7}$	24	242 †	8.2	51	59	36

* Average of two or more determinations

† Different plug size results in a plate/plug ratio of 60.2 instead of the usual ratio of 119.4 (see also Materials and Methods) being used in the calculation of M

** Number of locus revertants are given in parentheses.

TABLE 100 : Phenotypes of spores recovered from a cross of *mut5* to *mut7-1*, sporulated and germinated at 20°C.

Strain RO519-	Segregating alleles at *arg4*	*hom3*	Growth at after 36°C	Y	Designated mutator(s)	
1A	-	+	+	-	+	*mut5*
B	-	-	+	+	+	+
2A	-	-	+	-	+	*mut5*
B	+	+	+	+	+	+
3A	+	+	+	+	+	+
B	-	+	+	+	+	+
4A	+	-	-	+	*mut7*	+
B	+	+	+	+	+	+
C	-	-	+	-	+	*mut5*
5A	-	+	+	-	+	*mut5*
B	+	-	-	+	*mut7*	+
6A	+	+	+	-	+	*mut5*
B	-	-	+	-	+	*mut5*
7A	+	+	+	+	+	+
B	+	+	+	-	+	*mut5*
C	-	-	-	+	*mut7*	+
8A	-	-	+	-	+	*mut5*
B	-	+	-	+	*mut7*	+
9A	-	-	+	+	+	+
B	-	+	-	+	*mut7*	+
C	+	+	+	-	+	*mut5*
10A	-	-	+	+	+	+
B	-	+	-	+	*mut7*	+

TABLE 101: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1-11*, a revertant of *mut7-1*, sporulated and germinated at 20°C.

Strain RO520-	Segregating alleles at *arg4*	Segregating alleles at *hom3*	Growth at 36°C	Growth after γ	Designated mutator
1A	-	-	+	+	+
B	+	+	+	+	+
C	+	+	+	-	*mut5*
D	-	-	+	-	*mut5*
2A	+	-	+	-	*mut5*
B	-	-	+	-	*mut5*
C	+	+	+	+	+
D	-	+	+	+	+
3A	+	+	+	+	+
B	-	-	+	-	*mut5*
C	-	+	+	+	+
D	+	-	+	-	*mut5*
4A	-	-	+	-	*mut5*
B	+	-	+	-	*mut5*
C	-	+	+	+	+
5A	+	-	+	-	*mut5*
B	-	-	+	+	+
C	+	+	+	-	*mut5*
D	-	+	+	+	+
6A	+	+	+	+	+
B	-	+	+	+	+
C	-	-	+	-	*mut5*
D	+	-	+	-	*mut5*
7A	-	+	+	+	+
B	+	-	+	+	+
C	-	+	+	-	*mut5*
8A	+	-	+	-	*mut5*
B	+	+	+	-	*mut5*
C	-	-	+	+	+
D	-	+	+	+	+
9A	-	+	+	+	+
B	+	-	+	-	*mut5*
C	+	+	+	+	+
10A	+	-	+	-	*mut5*
B	-	+	+	+	+
C	-	-	+	-	*mut5*
D	+	+	+	+	+

TABLE 102: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1*, sporulated and germinated at 26°C

Strain RO521-	Segregating alleles at *arg4*	*hom3*	Growth at 36°C	after γ	Designated mutator	
1A	+	-	+	-	+	*mut5*
B	+	+	-	+	*mut7*	+
C	-	+	+	+	+	+
2A	-	-	+	+	+	+
B	+	+	+	+	+	+
3A	-	-	+	+	+	+
B	-	+	-	+	*mut7*	+
C	+	+	+	-	+	*mut5*
4A	-	+	+	-	+	*mut5*
B	-	-	-	+	*mut7*	+
C	+	+	+	+	+	+
5A	-	-	+	-	+	*mut5*
B	+	+	+	+	+	+
6A	+	+	+	-	+	*mut5*
B	-	+	-	+	*mut7*	+
C	+	-	+	+	+	+
7A	-	+	-	+	*mut7*	+
B	+	-	+	-	+	*mut5*
8A	+	+	+	+	+	+
B	-	-	+	+	+	+
9A	-	+	+	+	+	+
B	+	+	+	+	+	+
10A	-	+	+	-	+	*mut5*

TABLE 103: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1-11*, a revertant of *mut7-1*, sporulated and germinated at 26°C

Strain RO522-	Segregating alleles at *arg4*	*hom3*	Growth at 36°C	after γ	Designated mutator
1A	+	+	+	-	*mut5*
B	+	+	+	+	+
C	-	-	+	-	*mut5*
D	-	-	+	+	+
2A	+	-	+	-	*mut5*
B	-	+	+	+	+
C	+	-	+	-	*mut5*
D	-	+	+	+	+
3A	+	+	+	+	+
B	-	-	+	-	*mut5*
C	-	+	+	-	*mut5*
4A	-	-	+	-	*mut5*
B	+	-	+	-	*mut5*
C	+	+	+	+	+
D	-	+	+	+	+
5A	-	+	+	+	+
B	+	+	+	-	*mut5*
C	+	-	+	+	+
6A	-	+	+	+	+
B	-	-	+	-	*mut5*
C	+	+	+	+	+
D	+	-	+	-	*mut5*
7A	+	-	+	-	*mut5*
B	-	+	+	+	+
C	-	-	+	+	+
D	+	+	+	-	*mut5*
8A	-	-	+	-	*mut5*
B	-	+	+	-	*mut5*
C	+	+	+	+	+
D	+	-	+	+	+
9A	+	+	+	+	+
B	+	-	+	-	*mut5*
C	-	+	+	+	+
D	-	-	+	-	*mut5*
10A	+	+	+	-	*mut5*
B	-	-	+	+	+
C	+	+	+	+	+
D	-	-	+	-	*mut5*

TABLE 104: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1*, sporulated at 20°C and germinated at 26°C

Strain RO523-	Segregating alleles at *arg4*	*hom3*	Growth at after 36°C	γ	Designated mutator	
1A	+	-	+	-	+	*mut5*
B	+	+	-	+	*mut7*	+
C	-	+	+	+	+	+
2A	+	+	+	+	+	+
B	-	+	-	+	*mut7*	+
C	-	-	+	-	+	*mut5*
3A	-	+	-	+	*mut7*	+
B	+	-	+	-	+	*mut5*
C	+	+	-	+	*mut7*	+
4A	+	+	+	+	+	+
B	-	+	+	+	+	+
5A	+	-	+	-	+	*mut5*
B	-	+	-	+	*mut7*	+
6A	+	+	-	+	*mut7*	+
B	+	-	+	-	+	*mut5*
C	-	+	+	+	+	+
7A	-	-	+	+	+	+
B	+	-	+	-	+	*mut5*
8A	+	+	-	+	*mut7*	+
B	+	-	+	-	+	*mut5*
C	-	-	+	+	+	+
9A	+	+	-	+	*mut7*	+
B	-	-	+	+	+	+
C	-	-	+	-	+	*mut5*
10A	+	+	+	+	+	+

TABLE 105: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1-11*, a revertant of *mut7-1*, sporulated at 20°C and germinated at 26°C

Strain RO524-	Segregating alleles at *arg4*	*hom3*	Growth at 36°C	after γ	Designated mutator
1A	+	-	+	-	*mut5*
B	-	-	+	-	*mut5*
C	+	+	+	+	+
D	-	+	+	+	+
2A	-	-	+	+	+
B	-	+	+	+	+
C	+	+	+	-	*mut5*
3A	+	-	+	+	+
B	-	+	+	-	*mut5*
C	+	+	+	+	+
4A	+	+	+	+	+
B	-	-	+	-	*mut5*
C	-	+	+	+	+
D	+	-	+	-	*mut5*
5A	+	+	+	+	+
B	-	-	+	-	*mut5*
C	-	+	+	-	*mut5*
D	+	-	+	+	+
6A	+	+	+	-	*mut5*
B	-	-	+	+	+
C	-	+	+	+	+
D	+	-	+	-	*mut5*
7A	-	+	+	+	+
B	-	-	+	-	*mut5*
C	+	+	+	+	+
8A	-	+	+	+	+
B	+	-	+	-	*mut5*
C	+	-	+	-	*mut5*
D	-	+	+	+	+
9A	-	-	+	+	+
B	-	+	+	+	+
C	+	-	+	-	*mut5*
D	+	+	+	-	*mut5*
10A	+	-	+	-	*mut5*
B	+	-	+	-	*mut5*
C	-	+	+	+	+
D	-	+	+	+	+

TABLE 106: Phenotypes of spores recovered from a cross of *mut5* *mut7-1*, sporulated at 26°C and germinated at 20°C

Strain RO525-	Segregating alleles at *arg4*	*hom3*	Growth at 36°C	after Y	Designated mutator	
1A	-	+	+	+	+	+
B	-	-	+	+	+	+
2A	+	+	+	+	+	+
B	+	-	+	-	+	*mut5*
C	+	-	-	+	*mut7*	+
3A	+	+	+	-	+	*mut5*
B	+	-	-	+	*mut7*	+
C	-	+	+	+	+	+
4A	-	-	-	+	*mut7*	+
B	-	+	+	+	+	+
C	+	-	+	-	+	*mut5*
5A	-	+	+	+	+	+
B	+	+	+	+	+	+
6A	-	-	+	+	+	+
B	+	-	+	-	+	*mut5*
7A	+	+	+	+	+	+
8A	+	+	+	-	+	*mut5*
B	-	-	+	-	+	*mut5*
9A	+	+	+	-	+	*mut5*
B	-	-	+	+	+	+
C	-	-	-	+	*mut7*	+
10A	+	+	+	+	+	+
B	-	-	-	+	*mut7*	+
C	-	+	+	-	+	*mut5*

TABLE 107: Phenotypes of spores recovered from a cross of *mut5* to *mut7-1-11*, a revertant of *mut7-1*, sporulated at 26°C and germinated at 20°C.

Strain RO526-	Segregating alleles at *arg4*	*hom3*	Growth at after 36°C	Y	Designated mutator
1A	+	-	+	+	+
B	-	+	+	-	*mut5*
C	-	+	+	+	+
D	+	-	+	-	*mut5*
2A	-	+	+	+	+
B	-	-	+	-	*mut5*
C	+	+	+	+	+
3A	-	-	+	-	*mut5*
B	+	+	+	+	+
C	-	+	+	+	+
D	+	-	+	-	*mut5*
4A	+	-	+	-	*mut5*
B	-	+	+	+	+
C	+	-	+	+	+
D	-	+	+	-	*mut5*
5A	-	+	+	+	+
B	-	-	+	-	*mut5*
6A	+	+	+	+	+
B	+	-	+	-	*mut5*
C	-	-	+	+	+
7A	+	-	+	-	*mut5*
B	-	+	+	+	+
C	+	+	+	+	+
D	-	-	+	-	*mut5*
8A	-	-	+	-	*mut5*
B	+	+	+	+	+
C	-	+	+	+	+
D	+	-	+	-	*mut5*
9A	+	-	+	-	*mut5*
B	-	-	+	-	*mut5*
C	+	+	+	+	+
D	-	+	+	+	+
10A	-	+	+	+	+
B	-	-	+	-	*mut5*
C	+	-	+	-	*mut5*
D	+	+	+	+	+

TABLE 108: A summary of meiotic recombination between *hom3-10* and *mut5-1* in crosses heterozygous for *mut5-1*

mut genotype	Strain	PD	TT	NPD	Germinated at	Comments
5 / +	R065	4	3	0	26°C	
	R0520	6	4	0	20°C	contain the *mut7-1-11* revertant allele
	R0522	4	6	0	26°C	
	R0524	5	5	0	20°C	
	R0526	6	3	0	26°C	
$\frac{5}{+}\ \frac{+}{8}$	R0105	5	4	0	20°C	
		30	25	0		R = 0.23
$\frac{5}{+}\ \frac{+}{7}$	R085	10	5	0	26°C	
	R0504	8	2	1	26°C	
	R0519	2	7	0	20°C	
	R0521	2	6	0	26°C	
	R0523	4	4	0	20°C	
	R0525	1	7	1	26°C	
		27	31	2		R = 0.36
	R085	11	19	0	26°C	Germinated two weeks after sporulation was begun
$\frac{5}{+}\ \frac{7}{+}\ \frac{8}{+}$	R0113	11	13	0	20°C	
	R0118	2	7	0	20°C	
		13	20	0		R = 0.30
5 / +	R0520 522 524 526	21	18	0		contain the *mut7-1-11* revertant allele R = 0.23
$\frac{5}{+}\ \frac{+}{7}$	R0519 521 523 525	9	24	1		R = 0.44

mut5 spores from the cross involving both mutators).

To examine further the possibility that *mut7* was conferring enhanced recombination in strains also heterozygous for *mut5*, recombination between *hom3* and *mut5* was monitored in the above crosses. While there were no differences noted in recombination for different temperature within either the *mut7-1/mut5-1* or the *mut7-1-11/mut5-1* category, the total recombination for the first cross was 0.23 and for the latter cross was 0.44. The difference was significant ($\chi^2 = 5.34$, $P < 0.05$, corrected for continuity, for non-recombinant vs recombinant tetrads; NPD = two recombination events) between the two crosses. It would appear that the presence of *mut7* has resulted in a doubling of the map distance between *hom3* and *mut5*. This poses a question with respect to cross R0113 (Table 96): is *mut7* alone responsible for a generalized decrease in linkage between *mut5* and *hom3*? The linkage data from all crosses of *mut5* for *hom3* - *mut5* meiotic recombination are summarized in Table 108. Those crosses also heterozygous for *mut7* are usually enhanced for recombination, except in those cases where tetrads were dissected within four days of placing the diploids on sporulation medium (crosses R085 and 504; tetrads from cross R085 dissected two weeks after the first dissections (Table 92) had increased numbers of tetratype tetrads).

f. *mut6* (Tables 109-114)

Strains bearing *mut6* have no sensitivities to any mutagenic agents tested to date. The allele is considered to be variably dominant for the expression of enhanced *lys1-1* (suppressor) reversion.

Locus revertants of *lys1-1* are not enhanced in *mut6* strains. Lassie scores are about four times higher than *MUT*$^+$ in *mut6* haploids, for both *lys1-1* suppressor and *his1-7* revertants.

In my hands, the presence of *mut6* was correlated with enhanced petite production (see Appendix, Table A1). Normally, petite strains have enhanced *his1-7* reversion (Flury, von Borstel and Williamson, 1976). This was not the case for *mut6* haploid petites (Table A1). Stringent selection against ρ^- clones or clones with petite sectors resulted in a reduction in the variability of *mut6*-mediated dominance. This selection was accomplished by YG-replica plating of strains, or only picking bright red clones from YD medium, prior to a Lassie test.

In crosses with *mut7* and *mut8* the segregation of the *mut6* locus was detected by the presence of non-*ts* spore clones with enhanced suppressor mutations (where red locus revertants could be discerned). In agreement with Quah (unpublished data), detectable *lys1-1* locus revertant clones were not observed to be enhanced in frequency over that observed for *MUT*$^+$ strains on Lassie tests (Tables 113 and 114).

There appears to be no linkage between *mut7* and *mut6* (2P:19T:1N; Tables 109 and 110) or between *mut8* and *mut6* (4P:12T:2N; Tables 111 and 112). The enhanced numbers of revertants seen on limiting histidine in *MUT*$^+$ strains on the first page of Table 112 are due to fluctuations in the medium and not to mutator activity, as may be seen from the second page of the Table. The *mut6* allele does not confer enhanced reversion frequency at the allele *his1-1*.

Segregating *mut7 mut6* strains were indistinguishable from *mut7* strains or *mut6* strains for both *his1-7* and *lys1-1* reversion frequencies (Tables 109 and 110). It is likely that the double mutant may be distinguished by the reduction in *lys1-1* locus revertants compared to strains bearing only *mut7* (Table 113). This phenotype, together with the *mut7*-conferred *ts* phenotype was observed in the *mut7 mut6* spore clone RO86-7A (Tables 113 and 114), which segregated in a non-parental ditype (NPD) tetrad (2 *mut*: 2 MUT^+ with regard to mutator activity).

This apparent epistasis of *mut6* is seen again in the data shown in Table 114, for lysine prototrophs. Rates were not calculated for *his1-7* reversion in the double mutant due to the failure to isolate an easily confirmable *mut7 mut6 his1-7* strain in an NPD tetrad. Complementation tests were not performed for suspect double mutants from crosses RO85 and RO506 because of the ambiguity posed by *mut6* dominance.

Several segregants from *mut8/+*, *+/mut6* heterozygotes had high *his1-7* reversion frequencies reminiscent of *mut7 mut8* strains (Tables 111 and 112). Subsequent complementation tests of these (*his1-7*) mutators with *his1-7, mut8* testers confirmed that these were *mut8 mut6* double mutants. Furthermore, the non-complementing diploids (see Appendix, Table A2) had extremely high reversion rates for *his1-7/his1-7* relative to *mut8/mut8* diploid controls tested simultaneously. Hence, *mut6* may be dominant, not for the spontaneous mutations occurring in MUT^+ strains, but for those expressed in *mut8* strains.

TABLE 109: Phenotypes of spores recovered from cross RO86 ($\frac{mut7}{+} \times \frac{+}{mut6}$)

Strain RO86-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Growth at 36°C	Designated mutators
1A	-	1-7	41 (0)	55 (0)	-	*mut7*
B	-	1-7	66 (0)	60 (0)	+	+ *mut6*
2A°	+	1-1			+	+
B°	-	1-7	83 (4)	60 (0)	+	+ *mut6*
4A	-	1-7	52 (4)	30 (1)	-	*mut7*
B	+	1-1	0 (0)	52 (0)	+	+ *mut6*
5A	+	1-1	1 (0)	48 (0)	-	*mut7*
B	-	1-7	37 (2)	53 (0)	-	*mut7*
6A	+	1-1	0 (0)	54 (0)	-	*mut7*
B	-	1-7	32 (1)	33 (0)	-	*mut7*
7A	+	1-1	0 (0)	33 (0)	-	*mut7* *mut6*
B	-	1-7	7 (1)	16 (0)	+	+ +
C	-	1-7	8 (1)	15 (1)	+	+ +
D	+	1-1	0 (0)	27 (0)	-	*mut7* *mut6*
8A°	+	1-1	0 (0)	59 (0)	+	+ *mut6*
B	+	1-1	1 (0)	6 (0)	+	+ +
C	-	1-7	40 (3)	34 (0)	-	*mut7*
9A	+	1-7?	9 (0)	19 (0)	+	+ +
B	-	1-7	44 (2)	39 (0)	-	*mut7*
10A°					+	+
B	-	1-7	5 (1)	12 (0)	+	+ +
11A°					+	+
12A°					+	+
B	-	1-7	42 (4)	56 (0)	-	*mut7*
13A	+	1-1	0 (0)	36 (0)	-	*mut7*
B	-	1-7	33 (1)	45 (1)	-	*mut7*
14A	-	1-7	34 (0)	43 (0)	+	+ *mut6*
B	+	1-1	0 (0)	14 (0)	+	+ +
15A	-	1-7	45 (2)	42 (0)	-	*mut7*
B	-	1-7	11 (0)	7 (0)	+	+ +
16A	-	1-7	40 (2)	72 (1)	+	+ *mut6*
Bo	+	1-1	0 (0)	84 (0)	+	+ *mut6*
C	-	1-7	24 (2)	59 (0)	-	*mut7* +
D	+	1-1	0 (0)	52 (0)	-	*mut7* +
17A	-	1-7	52 (1)	57 (0)	-	*mut7*
B	+	1-1	1 (0)	47 (0)	-	*mut7*
19A	+	1-1	2 (0)	47 (0)	-	*mut7*
B	+	1-1	0 (0)	47 (0)	-	*mut7*
C°	-	1-7	30 (0)	40 (1)	+	+ *mut6*

TABLE 109: (continued)

Strain RO86-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Growth at 36°C	Designated mutator	
21A	-	1-7	25	(21)	43	(0)	-	*mut7*	
B	+	1-1	1	(0)	13	(0)	+	+	+
C	+	1-1	0	(0)	66	(0)	+	+	*mut6*
22A	+	1-1	0	(0)	13	(0)	+	+	+
B	-	1-7	38	(1)	29	(0)	-	*mut7*	
23A	-	1-7	18	(0)	52	(0)	+	+	*mut6*
B	+	1-1	0	(0)	52	(0)	+	+	*mut6*
24A	+	1-1	1	(1)*	40	(1)	-	*mut7*	
B	-	1-7	8	(0)	11	(0)	+	+	+
C$^{\rho}$	-	1-7	20	(0)	31	(0)	+	+	
26A$^{\rho}$	+	1-1	0	(0)	82	(0)	+	+	*mut6*
B	+	1-1	0	(0)	25	(0)	+	+	
C	-	1-7	21	(4)	30	(0)	-	*mut7*	
D	-	1-7	65	(2)	40	(0)	-	*mut7*	
27A	+	1-1	0	(0)	11	(0)	+	+	+
B$^{\rho}$	-	1-7	27	(0)	21	(0)	+	+	
28A	+	1-1	0	(0)	42	(0)	-	*mut7*	
B	-	1-7	37	(0)	54	(0)	-	*mut7*	
31A	-	1-7	21	(1)	44	(0)	-	*mut7*	
B	-	1-7	54	(4)	47	(0)	-	*mut7*	
32A	-	1-7	8	(0)	10	(0)	+	+	+
B$^{\rho}$	+	1-1	3	(0)	31	(0)	+	+	
C	+	1-1	0	(0)	29	(0)	-	*mut7*	
D	-	1-7	20	(1)	29	(0)	-	*mut7*	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.

ρ spores failed to grow when replica-plated to YG medium.

TABLE 110: Phenotypes of spores recovered from cross RO506 ($\frac{mut7}{+}$ $\frac{+}{mut6}$)

Strain RO506-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth at 36°C	Designated mutator(s)	
1A	-	1-7	26,38 (1)	52,43 (0)	-	*mut7*	
B	+	1-7	0,1 (0)	18,40 (12)	-	*mut7*	
C	-	1-7	7,15 (2)	14,7 (0)	+	+	+
D	+	1-1	0,1 (0)	42,33 (1)	+	+	*mut6*
2A	-	1-7	26,41 (0)	44,53 (1)	+	+	*mut6*
B	-	1-7	7,13 (0)	18,13 (0)	+	+	+
C	+	1-1	0,0 (0)	43,34 (0)	-	*mut7*	
D	+	1-1	0,1 (0)	28,32 (0)	-	*mut7*	
3A	+	1-1	0,0 (0)	41,52 (1)	-	*mut7*	
B	+	1-1	0,1 (0)	29,39 (0)	-	*mut7*	
C	-	+		41,39 (0)	+	+	*mut6*
D	-	1-7	3,17 (1)	17,17 (0)	+	+	+
4A	-	1-7	28,29 (1)	39,32 (0)	-	*mut7*	
B	+	1-1	0,0 (0)	31,42 (0)	-	*mut7*	
C	-	1-7	19,17 (0)	13,7 (0)	+	+	+
D	+	1-1	0,1 (0)	68,61 (0)	+	+	*mut6*
5A	+	1-1	0,0 (0)	18,13 (0)	+	+	+
B	-	1-7	23,35 (5)	44,44 (0)	-	*mut7*	
C	-	1-7	45,43 (2)	69,43 (0)	+	+	*mut6*
D	+	1-1	0,2 (0)	32,31 (0)	-	*mut7*	
6A	+	1-1	0,0 (0)	8,11 (0)	+	+	+
B	-	1-1	0,0 (0)	41,39 (0)	-	*mut7*	
C	-	1-7	39,33 (2)	39,45 (0)	-	*mut7*	
D	+	1-1	17,37 (17)	47,47 (0)	+	+	*mut6*
7A	+	1-1	0,0 (0)	43,42 (1)	+	+	6
B	-	1-7	18,10 (1)	17,21 (0)	+	+	+
C	+	1-1	0,1 (0)	31,41 (0)	-	*mut7*	
D	-	1-7	38,34 (7)	40,30 (0)	-	*mut7*	
8A	+	1-1	1,0 (0)	55,31 (1)	-	*mut7*	
B	-	1-7	24,47 (8)	32,52 (0)	-	*mut7*	
C	-	1-7	28,41 (2)	44,34 (1)	+	+	*mut6*
D	+	1-1	1,1 (0)†	16,17 (1)	+	+	+
9A	+	1-1	0,1 (0)	47,45 (0)	-	*mut7*	
B	-	1-7	24,46 (2)	42,32 (0)	+	+	*mut6*
C	-	1-7	92,108(1)†	42,34 (0)	-	*mut7*	
D	+	1-1	0,0 (0)†	13,14 (0)	+	+	+
10A	-	1-7	41,39 (0)	60,46 (0)	+	+	*mut6*
B	+	1-1	0,0 (0)	15,15 (0)	+	+	+
C	+	1-1	2,2 (0)†	46,53 (0)	-	*mut7*	
D	-	1-7	16,31 (0)†	28,46 (0)	-	*mut7*	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. † data from another experiment.

TABLE 111: Phenotypes of spores recovered from cross RO106 ($\frac{mut8}{+}$ $\frac{+}{mut6}$)

Strain RO106-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Designated mutator(s)	
1A	+	1-1	2,4 (0)	67,56 (0)		*mut6*
B	-	1-7	107,129(85)	26,18 (1)	*mut8*	+
C	-	1-7	60,55 (0)	60,65 (0)	+	*mut6*
D	+	1-1	1,1 (0)	34,23 (24)		+
2A	-	1-1	1,4 (0)	46,35 (0)		*mut6*
B	+	1-7	16,15 (1)	14,8 (0)	+	+
3A	-	1-7	123,136(58)	26,17 (0)	*mut8*	+
B	+	1-1	1,0 (0)	41,52 (2)		*mut6*
C	-	1-7	14,11 (3)	14,10 (0)	+	+
4A	+	1-1	0,0 (0)	11,12 (0)	+	+
B	+	1-7	144,93 (73)	33,44 (0)	*mut8*	+
C	-	1-7	21,33 (0)	46,47 (0)	+	*mut6*
D	-	1-1	2,3 (0)	38,33 (2)	*mut8*	*mut6*
5A	+	1-1	2,6 (0)	20,16 (0)	*mut8*	+
B	-	1-7	28,37 (3)	39,48 (0)	+	*mut6*
C	-	1-7	1052,1143(703)	22,22 (0)	*mut8*	*mut6*
6A	-	1-7	3,2 (2)	9,5 (0)	+	+
B	-	1-7	29,19 (0)	60,57 (0)	+	*mut6*
7A	+	1-1	3,2 (0)	31,27 (0)	*mut8*	*mut6*
B	-	1-7	13,7 (0)	21,11 (0)	+	+
C	-	1-7	6,9 (2)	14,14 (1)	+	+
D$^{\rho}$	+	1-1	3,1 (0)	16,39 (1)	*mut8*	*mut6*
8A$^{\rho}$	-	1-7	632,595(96)	22,25 (0)	*mut8*	*mut6*
B	+	1-1	0,0 (0)	60,31 (0)	+	*mut6*
C	-	1-7	21,25 (0)	11,8 (0)	+	+
D	+	1-1	3,5 (0)	20,10 (1)	*mut8*	+
9A	+	1-1	0,1 (0)	37,33 (3)	+	*mut6*
B	-	1-7	25,34 (1)	16,16 (1)	+	+
10A$^{\rho}$	-	1-7	738,807(347)	19,18 (0)	*mut8*	*mut6*
B	-	1-7	58,53 (16)	53,39 (0)	+	*mut6*
C	+	1-1	1,1 (0)	20,19 (0)	*mut8*	+
D	+	1-1	0,0 (0)	12,10 (0)	+	+
12A	+	1-1	0,0 (0)	23,20 (0)		+
B	+	1-1	1,1 (0)	52,30 (0)		*mut6*
C	-	1-7	55,57 (1)	34,41 (0)	+	*mut6*
D	-	1-7	70,48 (48)	21,17 (0)	*mut8*	+
14A	+	1-1	2,0 (0)	10,20 (0)	*mut8*	+
B	-	1-7	27,20 (0)	46,48 (0)	+	*mut6*
C	+	1-1	2,3 (0)	21,23 (0)	*mut8*	+
D	-	1-7	33,46 (0)	82,47 (0)	+	*mut6*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

ρ Spores fail to grow when replica-plated to YG medium.

TABLE 112: Phenotypes of spores recovered from cross RO512($\frac{mut8}{+}$ $\frac{+}{mut6}$)

Strain RO512-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Designated mutator(s)
1A	+	1-1	2,0 (0)	32,32 (1)	+ *mut6*
B	-	1-7	304,299(61)	15,14 (0)	*mut8* +
C	+	1-7	1190,1090(61)	30,21 (0)	*mut8* *mut6*
D	-	1-1	1,0 (0)	17,12 (0)	+ +
2A	+	1-1	0,0 (0)	22,13 (0)	+ +
B	-	1-7	187,219(19)	21,8 (0)	*mut8* +
C	+	1-1	2,3 (0)	17,17 (0)	*mut8* *mut6*
D	-	1-7	123,135(1)	39,46 (0)	+ *mut6*
3A	+	1-1	1,0 (0)	19,14 (0)	+ +
B	+	1-1	0,1 (0)	29,46 (0)	+ *mut6*
C	-	1-7	1243,1126(205)	26,21 (0)	*mut8* *mut6*
D	-	1-7	300,218(54)	12,23 (0)	*mut8* +
4A	+	1-1	0,1 (0)	43,41 (1)	+ *mut6*
B	-	1-7	80,71 (0)	47,34 (0)	+ *mut6*
C	+	1-1	2,5 (0)	9,17 (1)	*mut8* +
D	-	1-7	357,299(66)	30,22 (2)	*mut8* +
5A	-	1-7	154,141(0)	41,46 (0)	+ *mut6*
B	+	1-1	1,0 (0)	19,9 (2)	*mut8* *mut6*
C	+	1-1	2,4 (0)	18,27 (0)	*mut8* +
D	-	1-7	67,45 (3)	7,12 (0)	+ +
6A	+	1-1	0,1 (0)	16,14 (0)	+ +
B	+	1-1	5,3 (0)	31,35 (1)	*mut8* *mut6*
C	-	1-7	293,312(60)	15,24 (0)	*mut8* +
D	-	1-7	74,91 (0)	32,41 (0)	+ *mut6*
7A	+	1-1	0,0 (0)	45,54 (0)	+ *mut6*
B	-	1-7	97,73 (0)	43,44 (2)	+ *mut6*
C	-	1-7	266,246(54)	20,24 (0)	*mut8* +
D	+	1-1	2,4 (0)	22,15 (0)	*mut8* +
8A	+	1-1	1,0 (0)	33,39 (0)	+ *mut6*
B	-	1-7	82,71 (1)	43,53 (0)	+ *mut6*
C	+	1-1	3,2 (0)	19,29 (0)	*mut8* +
D	-	1-7	247,190(22)	22,26 (0)	*mut8* +
9A	-	1-7	73,47 (0)	12,8 (0)	+ +
B	-	1-7	905,923(107)	15,16 (0)	*mut8* *mut6*
C	+	1-1	1,1 (0)	27,32 (0)	*mut8* *mut6*
D	+	1-1	1,0 (0)	4,11 (0)	+ +
10A	+	1-1	3,0 (0)	32,25 (0)	*mut8* *mut6*
B	-	1-7	190,219(70)	16,20 (0)	*mut8* +
C	-	1-7	72,83 (0)	17,9 (0)	+ +
D	+	1-1	0,0 (0)	46,33 (0)	+ *mut6*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 112: (continued--experiment #2)

Strain RO512-	Prototrophs arising on limiting histidine		Designated *mut(s)*	
1A	1,0	(0)	+	*6*
B	188,195	(58)	*8*	+
C			*8*	*6*
D	0,0	(0)	+	+
2A	0,1	(0)	+	+
B	99,110	(176)	*8*	+
C			*8*	*6*
D	30,28	(1)	+	*6*
3A	0,0	(0)	+	+
B	1,1	(0)	+	*6*
C	1066,889	(153)	*8*	*6*
D	209,253	(179)	*8*	+
4A	1,0	(0)	+	*6*
B	37,19	(2)	+	*6*
C	5,5	(0)	*8*	+
D	1226,1207	(1192)*	*8*	+
5A	41,28	(1)	+	*6*
B	1,0	(0)	*8*	*6*
C	6,1	(0)	*8*	+
D	6,11	(0)	+	+
6A	1,0	(0)	+	+
B			*8*	*6*
C	251,265	(70)	*8*	+
D	25,25	(0)	+	*6*
7A	2,0	(0)	+	*6*
B	35,34	(0)	+	*6*
C	197,174	(55)	*8*	+
D	4,2	(0)	*8*	+
8A	0,0	(0)	+	*6*
B	39,20	(0)	+	*6*
C	6,8	(1)	*8*	+
D	161,185	(33)	*8*	+
9A	13,18	(35)	+	+
B			*8*	*6*
C	1,1	(0)	*8?*	*6?*
D	0,0	(0)	+	+
10A	2,2	(0)	*8*	*6*
B	165,173	(43)	*8*	+
C	15,9	(2)	+	+
D	1,0	(0)	+	*6*

* *'Jackpot'* (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 113: Presumptive *lys1-1* locus (red) revertants arising on limiting lysine in haploid strains segregating for *mut6* *mut7* or *mut8*

Strain RO86-	Designated *mut*		Red Revertants
1A	7	?	28
5A	7	?	23
6A	7	?	30
B	7	?	8
7A	7	6	6
10B	+	+	1
15A	7	?	15
16A	+	6	6
C	7	+	21
24A	7	?	17
26D	7	?	16
28B	7	?	17
32C	7	?	1
RO106-			
3A	8	+	1,1
C	+	+	1,1
4A	+	+	3,1
B	8	+	4,5
5A	8	+	5,3
9B	+	+	1,3
10C	8	+	3,1
12A	8	+	2,0
14C	8	+	1,2

Strain RO506-	Designated *mut*		Red Revertants
1A	7	?	7,7
2B	+	+	1,0
D	7	?	5,0
3A	7	?	16,18
D	+	+	0,1
4C	+	+	1,0
5A	+	+	2,0
6C	7	?	2,4
7A	+	6	1,0
B	+	+	1,2
8D	+	+	3,2
10B	+	+	4,0

TABLE 114: Mutation rates (M) of strains from crosses RO86, RO506, RO106, and RO512 in terms of (unreverted) cells per Lassie test plate

Mutator(s)	RO strain	*his1* allele	Lysine Lassie score*	Cells/plug* $\times 10^{-4}$	M_{lys} $\times 10^{8}$	Histidine Lassie score*	Cells/plug* $\times 10^{-4}$	M_{his} $\times 10^{8}$
mut6	506-9B	1-7	35	178	8.2	38	126	13
	106-1C	"	64(1)	96	28	28	104	11
	-4C	"	47	152	13	36	116	13
	-5A	"	44	92	20	26	86	13
	506-4D	1-1	59	137	18	0	117	< 0.4
	106-1D	"	62	154	17	0	157	< 0.3
	-8B	"	44	160	12	1	107	0.4
mut7	86-16C	1-7	35(10)	91§	16	37	103§	15
mut8	106-1B	1-7	25(1)	150	7.0	101	129	33
	-4B	"	27(5)	172	6.6	94	146	27
	-5A	1-1	20(9)	210	4.0	3	122	1.0
mut6mut7	86-7A	1-1	38(1)	102§	16	0	68§	< 0.6
mut6mut8	106-5C	1-7	26	190§	5.7	740	149	208
	-4D	1-1	37	162§	9.6	2	113	0.7
	512-5B	"	9	55§	6.8	0	72	< 0.6
	-6B	"	46	181	11	4	89	1.9
	-10A	"	45(3)	177	11	4	76	2.2
MUT^{+}	86-7B	1-7				9	114	3.3
	106-8C	"	15	171	3.7	17	130	5.5
	-4A	1-1	9	143	2.6	0	121	< 0.4

* Average of two determinations. Numbers in parentheses are lysine locus revertants
§ Average of four determinations

This *mut6 mut8*-mediated mutator phenotype was confirmed by data shown in Table 114. Oddly enough, the double mutant *mut8 mut6* has low rates of reversion for *his1-1* and *lys1-1*, including *lys1-1* locus revertants.

g. *mut9* (Tables 115-121)

An allele of *mut8* (designated *mut8-2*) is present in the *mut9* strain RO79-8A (see section B2). Crosses RO89 and RO508 confirmed that there was a *mut8* allele segregating from *mut9*-mediated sensitivity to γ -irradiation (Tables 115 and 117), as evidenced by the synergistic enhancement of *his1-7* reversion (strains RO89-1A and RO508-1D), of *his1-1* reversion (RO89-2C and RO508-2A) and of *lys1-1* locus reversion (Table 120; strains RO508-1D and 2A) observed in several *mut7* spore clones.

The *mut9* allele has been assumed to co-segregate with sensitivity to γ -irradiation in this study. The *mut9*, presumptive non-*mut8-2* (based on low *his1-1* Lassie scores) strain RO89-2D was crossed to a *mut7* strain, and the resulting diploid, RO507 segregated only for *mut7* and *mut9* (Table 16). It may be concluded from the data shown in Table 16, as well as that from Tables 115 and 117, that *mut7 mut9* strains have drastically reduced viability. Six presumptive *mut9 mut7* spores failed to form colonies. Recoverable "double mutant" spore clones were often reverted for either *mut7* or for *mut9*, or were contaminants (see RO507-7A or 11C, Table 116). Lassie tests of viable *mut7 mut9* strains indicated that both *his1-7* and *lys1-1* reversion frequencies were reduced in these strains. The third cross of *mut7* to a *mut9 mut8*

TABLE 115: Phenotypes of spores recovered from cross RO89 ($\frac{mut7}{+}$ $\frac{+}{mut9}$)

Strain RO89-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival after γ	Designated mutators
1A	-	1-7	495 (67)	92 (5)	-	+	*7* + *8*
B	-	1-7	34 (25)	17 (0)	+	+	+ + *8*
C	+	1-1	2 (0)	16 (0)	+	+	+ + +
D†	+	1-1	0,0 (0)	12,24 (43)	-	-	*7* *9* +
2A	-	1-7	33 (2)	48 (0)	-	+	*7* + +
B	-	1-7	70,66 (54)	80 (0)	+	-	+ *9* *8*
C	+	1-1	44 (34)	79 (0)	-	+	*7* + *8*
D	+	1-1	0 (0)	92 (0)	+	-	+ *9* +
3A	+	1-1	0 (0)	30 (0)	-	+	*7* + +
B	+	1-1	2 (0)	14 (0)	+	+	+ + +
C	-	1-7	65 (29)	79 (2)	+	-	+ *9* *8*
D†	-	1-7	217,210 (34)	58,28 (12)	-	-	*7* *9* *8*
4A	+	1-1	1 (0)	33 (1)	-	+	*7* + +
B	-	1-7	31 (1)	39 (2)	-	+	*7* + +
C	+	1-1	7 (0)	80 (11)	+	-	+ *9* *8*
D	-	1-7	92 (13)	95 (0)	+	-	+ *9* *8*
5A	+	1-1	0 (0)	9 (0)	+	+	+ + +
B	+	1-1	0 (0)	11 (0)	+	+	+ + +
C† D†	+		79,112 (109)*	19,20 (0)	+	+	
6A	+	1-1	7 (0)	71 (0)	+	-	+ *9* *8*
B	+	1-1	1 (0)	34 (0)	-	+	*7* + +
C	-	1-7	53 (69)	70 (1)	+	-	+ *9* *8*
D	-	1-7	40 (2)	41 (1)	-	+	*7* + +
7A	+	1-1	4 (0)	89 (30)	+	-	+ *9* *8*
B	-	1-7	3 (2)	8 (0)	+	+	+ + +
C	+	1-1	1 (0)	33 (0)	-	+	*7* + +
D†	-	1-7	244,199 (90)	53,33 (0)	-	-	*7* *9* *8*
8A	-	1-7	55 (1)	42 (1)	-	+	*7* + +
B	-	1-7	36 (4)	33 (0)	-	+	*7* + +
C	+	1-1	7 (0)	42 (1)	+	-	+ *9* *8*
D	+	1-1	3 (0)	67 (6)	+	-	+ *9* *8*

TABLE 115: (continued)

Strain RO89-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine		lysine		Survival at 36°C	after γ	Designated mutators		
9A	-	1-7	52	(16)	40	(0)	-	+	*7*	+	+
B	+	1-1	6	(0)	90	(7)	+	-	+	*9*	*8*
C	+	1-1	0	(0)	11	(0)	+	+	+	+	+
D†	+		66,91	(5)	75,32	(183)	+	-			
10A	+	1-1	1	(0)	33	(1)	-	+	*7*	+	+
B	-	1-7	33	(10)	24	(0)	+	+	+	+	*8*
C	+	1-1	1	(0)	73	(0)	+	-	+	*9*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† Semi-lethal spores were recovered as 'pinpoint' colonies. Several of these show unexpected phenotypes (see text for further discussion).

TABLE 116: Phenotypes of spores recovered from cross RO507($\frac{mut7}{+}$ $\frac{+}{mut9}$)

Strain RO507-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Survival at 36°C	Survival after γ	Designated mutator(s)	
1A	-	1-7	58,64 (2)	97,163(0)	+	-	+	*mut9*
B	+	1-1	1,2 (0)	53,28 (0)	-	+	*mut7*	+
C	-	1-7	80,84 (3)	113,152(8)	+	-	+	*mut9*
D	+	1-1	2,1 (0)	65,53 (0)	-	+	*mut7*	+
2A	+	1-1	4,0 (0)	165,150(2)	+	-	+	*mut9*
B	-	1-7	40,25 (1)	4,6 (0)	-	-	*mut7*	*mut9*
C	-	1-7	12,12 (1)	9,12 (7)	+	+	+	+
D	+	1-1	1,1 (0)	56,46 (0)	-	+	*mut7*	+
3A	+	1-1	2,0 (0)	42,51 (1)	-	+	*mut7*	+
B	-	1-7	95,120(0)	165,173(0)	+	-	+	*mut9*
C	-	1-7	66,48 (5)	71,58 (0)	-	+	*mut7*	+
D	+	1-1	3,3 (0)	161,181(0)	+	-	+	*mut9*
4A	-	1-7	77,64 (2)	172,120(6)	+	-	+	*mut9*
B	-	1-7	78,74 (4)	73,69 (0)	-	+	*mut7*	+
C	+	1-1	0,1 (0)	19,11 (0)	+	+	+	+
D†								
5A	+	1-7	52,59 (1)	48,44 (0)	-	+	*mut7*	+
B	-	1-7	76,95 (2)	215,237(1)	+	-	+	*mut9*
C	+	1-1	0,4 (0)	54,56 (0)	-	+	*mut7*	+
D	-	1-1	1,2 (0)	148,166(52)	+	-	+	*mut9*
6A	+	1-1	0,0 (0)	17,19 (0)	+	+	+	+
B	-	1-7	14,23 (1)	20,19 (0)	+	+	+	+
C†								
7A†	-	1-7	18,21 (0)	14,20 (0)	+	+	+	+
B	-	1-7	16,18 (7)	8,19 (0)	+	+	+	+
C†	+	1-1	1,0 (0)	217,213(193)*	-	-	*mut7*	*mut9*
D	+	1-1	0,0 (0)	21,17 (0)	+	+	+	+
8A	+	1-1	3,2 (0)	66,34 (0)	-	+	*mut7*	+
B	+	1-1	1,0 (0)	17,16 (0)	+	+	+	+
C	-	1-7	78,82 (3)	184,158(3)	+	-	+	*mut9*
9A	-	1-7	96,105(1)	136,173(3)	+	-	+	*mut9*
B	-	1-7	51,50 (4)	45,39 (0)	-	+	*mut7*	+
C	+	1-1	1,0 (0)	8,18 (0)	+	+	+	+
10A	+	1-1	1,2 (0)	166,169(0)	+	-	+	*mut9*
B	-	1-7	51,63 (3)	57,54 (0)	-	+	*mut7*	+
C	-	1-7	10,12 (0)	11,7 (0)	+	+	+	+
11A	-	1-7	7,15 (0)	14,16 (0)	+	+	+	+
B	-	1-7	83,97 (8)	193,228(0)	+	-	+	*mut9*
C†	+	1-1	0,1 (0)	44,37 (0)	-	+	*mut7*	+
D	+	1-1	0,2 (0)	59,57 (0)	-	+	*mut7*	+

**,* see previous table footnote. † semi-lethal,'pinpoint' colony observed.

TABLE 117: Phenotypes of spores recovered from cross RO508 ($\frac{mut7}{+}$ $\frac{+}{mut9}$)

Strain RO508-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	after γ	Designated mutator(s)
1A	+	1-1	1,0 (0)	13,15 (0)	+	+	+ + +
B	-	1-7	99,87 (48)	164,166(1)	+	-	+ 9 8
C	+	1-1	0,0 (0)	42,61 (12)	-	-	7 9 +
D	-	1-7	535,466(14)	100,110(0)	-	+	7 + 8
2A	+	1-1	57,64 (1)	91,100(5)	-	+	7 + 8
B	+	1-1	5,3 (0)	109,104(0)	+	-	+ 9 8
C	-	1-7	20,20 (0)	42,26 (29)*	-	-	7 9 +
D	-	1-7	9,12 (0)	15,12 (0)	+	+	+ + +
3A	-	1-7	42,50 (4)	57,56 (0)	-	+	7 + +
B	+	1-1	1,0 (0)	8,15 (0)	+	+	+ + +
C	+	1-1	11,5 (0)	49,54 (7)	-	-	7 9 8
D	-	1-7	81,93 (28)	59,71 (0)	+	-	+ 9 8
4A	+	1-1	5,6 (0)	104,83 (1)	+	-	+ 9 8
B	-	1-7	17,11 (0)	13,15 (0)	+	+	+ + +
C	-	1-7	74,55 (4)	12,10 (0)	-	-	7 9
D	+	1-1	2,0 (0)	40,53 (0)	-	+	7 + +
5A	+	1-1	2,0 (0)	73,97 (9)	+	-	+ 9 +
B	-	1-7	33,31 (1)	46,42 (0)	-	+	7 + +
C	+	1-1	14,7 (0)	58,54 (0)	-	-	7 9 8
D	-	1-7	37,32 (54)	14,20 (0)	+	+	+ + 8
6A	-	1-7	32,43 (1)	40,44 (0)	-	+	7 + +
B	+	1-1	0,1 (0)	7,13 (0)	+	+	+ + +
C	-	1-7	112,98 (26)	127,78 (16)	+	-	+ 9 8
D	+	1-1	7,9 (0)	39,34 (7)	-	-	7 9 8
7A	-	1-7	25,31 (35)	17,22 (0)	+	+	+ + 8
B	+	1-1	1,0 (0)	9,12 (0)	+	+	+ + +
C	+	1-1	4,5 (0)	28,32 (1)	-	-	7 9 +
D	-	1-7	486,449(20)	1529,1520 + (1552)*	-	-	7 9 8
8A	-	1-7	74,83 (25)	764,716(698)*	+	-	+ 9 8
B	+	1-1	9,1 (0)	85,116(2)	+	-	+ 9 8
C	+	1-1	1,0 (0)	39,33 (0)	-	+	7 + +
D	-	1-7	50,43 (2)	47,60 (0)	-	+	7 + +
9A	-	1-7	13,9 (0)	15,13 (0)	+	+	+ + +
B	-	1-7	276,181(339)	47,68 (6)	-	-	7 9 8
C	+	1-1	0,2 (0)	11,12 (1)	+	+	+ + +
D	+	1-1	9,8 (0)	8,13 (0)	-	-	7 9 8
10A	-	1-7	31,33 (0)	18,26 (0)	-	-	7 9 +
B	-	1-7	179,168(34)	46,55 (0)	-	-	7 9 8
C	+	1-1	1,1 (0)	13,15 (0)	+	+	+ + +
D	+	1-1	4,11 (0)	16,15 (0)	+	+	+ + 8

** Pre-existing prototrophs are parenthesized.
* 'Jackpot' (unparenthesized numbers include pre-existing prototrophs)

TABLE 118: Phenotypes of spores recovered from cross RO109($\frac{mut8}{+}$ $\frac{+}{mut9}$)

Strain RO109-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth after γ	Designated mutator(s)
1A	-	1-7	95,115(2)	178,172(1)	-	+ *mut9*
B	+	1-1	1,4 (0)	99,98 (1)	-	+ *mut9*
C	+	1-1	7,5 (0)	21,19 (0)	+	*mut8* +
D	-	1-7	181,159(152)	30,26 (0)	+	*mut8* +
2A	-	1-7	55,75 (5)	95,138(0)	-	+ *mut9*
B	+	1-1	4,5 (0)	20,21 (0)	+	*mut8* +
C	+	1-1	2,2 (0)	107,132(11)	-	+ *mut9*
D	-	1-7	136,176(71)	24,24 (0)	+	*mut8* +
3A	-	1-7	92,225(113)	17,21 (0)	+	*mut8* +
B	+	1-1	1,1 (0)	166,99 (1)	-	+ *mut9*
C	-	1-7	136,84 (160)	26,32 (0)	+	*mut8* +
D	+	1-1	0,0 (0)	108,97 (3)	-	+ *mut9*
4A	+	1-1	0,2 (0)	135,118(7)	-	+ *mut9*
B	-	1-7	68,91 (15)	116,129(5)	-	+ *mut9*
C	-	1-7	112,171(176)	22,18 (0)	+	*mut8* +
D	+	1-1	3,4 (0)	27,28 (0)	+	*mut8* +
5A†	+		107,60 (105)	15,14 (2)	+	
B	-	1-7	126,94 (203)	24,24 (1)	+	*mut8* +
C	-	1-7	79,72 (1)	194,134(0)	-	+ *mut9*
D	+	1-1	3,1 (0)	134,118(7)	-	*mut9*
6A	-	1-1	6,3 (0)	120,107(1)	-	*mut9*
B	+	1-1	1,0 (0)	10,13 (0)	+	+
C	-	1-7	63,90 (7)	148,122(1)	-	+ *mut9*
D	+	1-7	184,114(73)	23,23 (0)	+	*mut8* +
7A	-	1-7	60,73 (5)	146,95 (1)	-	+ *mut9*
B	-	1-7	108,128(238)	17,24 (0)	+	*mut8* +
C	+	1-1	0,0 (0)	142,186(1)	-	+ *mut9*
D	+	1-1	3,1 (0)	23,25 (1)	+	*mut8* +
8A	-	1-7	124,85 (78)	18,23 (2)	+	*mut8* +
B	+	1-1	2,0 (0)	131,148(18)	-	+ *mut9*
C	+	1-1	7,3 (0)	23,28 (0)	+	*mut8* +
D	-	1-7	83,83 (2)	174,138(2)	-	+ *mut9*
9A	+	1-7	53,71 (0)	117,125(0)	-	+ *mut9*
B	+	1-1	5,5 (0)	20,23 (0)	+	*mut8* +
10A	-	1-7	185,225(85)	109,94 (0)	-	*mut8 mut9*
B	+	1-1	1,0 (0)	137,149(2)	-	+ *mut9*
C	-	1-7	11,14 (0)	15,18 (0)	+/-	+ +

TABLE 118: (continued)

Strain RO109-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth after γ	Designated mutator(s)
11A	+	1-1	4,6 (0)	14,21 (1)	+	*mut8* +
B	+	1-1	1,1 (0)	17,18 (1)	+	+
C	-	1-7	85,78 (8)	81,150(1)	-	+ *mut9*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

† This strain failed to mate. The phenotype is virtually identical to a heteroallelic (at *his1*) diploid's.

TABLE 119: Phenotypes of spores recovered from cross RO514($\frac{mut8}{+}$ $\frac{+}{mut9}$)

Strain RO514-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Growth after γ	Designated mutator(s)	
1A	All are		98,120(15)	71,82 (0)	-	*mut8*	*mut9*
B	*hom3*	*his1-7*	98,145(23)	18,33 (0)	+	*mut8*	+
C			127,103(10)	107,99 (0)	-	*mut8*	*mut9*
D			99,99 (8)	22,19 (0)	+	*mut8*	+
2A			95,91 (65)	27,19 (1)	+	*mut8*	+
B			94,68 (42)	67,73 (1)	-	*mut8*	*mut9*
C			81,56 (54)	21,16 (0)	+	*mut8*	+
D			88,109(32)	93,80 (5)	+	*mut8*	
3A			140,144(47)	21,25 (0)	+	*mut8*	+
B			43,47 (48)	12,16 (2)	+	*mut8*	+
C			59,57 (25)	90,82 (1)	-	*mut8*	*mut9*
D			145,80 (15)	107,92 (20)	-	*mut8*	*mut9*
4A			104,101(18)	80,107(1)	-	*mut8*	*mut9*
B			99,95 (23)	19,27 (0)	+	*mut8*	+
C			70,85 (39)	114,88 (18)	-	*mut8*	*mut9*
D			122,91 (20)	15,33 (1)	+	*mut8*	+
5A			110,155(53)	23,14 (0)	+	*mut8*	+
B			64,49 (21)	70,104(0)	-	*mut8*	*mut9*
C			21,19 (23)	13,17 (0)	+		+
D			111,100(13)	64,83(1)	-	*mut8*	*mut9*
6A			95,60 (57)	19,24 (0)	+	*mut8*	+
B			68,57 (16)	84,92 (2)	-	*mut8*	*mut9*
C			89,94 (16)	104,91 (0)	-	*mut8*	*mut9*
D			131,160(55)	19,33 (1)	+	*mut8*	+
7A			69,60 (14)	95,80 (2)	-	*mut8*	*mut9*
B			118,132(35)	21,30 (4)	+	*mut8*	+
C			121,85 (28)	74,93 (0)	-	*mut8*	*mut9*
D			85,75 (32)	16,31 (0)	+	*mut8*	+
8A			92,99 (13)	97,78 (0)	-	*mut8*	*mut9*
B			125,97 (14)	77,91 (1)	-	*mut8*	*mut9*
C			91,75 (21)	28,25 (1)	+	*mut8*	+
D			93,103(17)	1155,1044(1195)*	+	*mut8*	+
9A			119,160(28)	87,112(5)	-	*mut8*	*mut9*
B			28,35 (30)	14,14 (1)	+	*mut8*	+
C			69,56 (25)	27,20 (0)	+	*mut8*	+
D			112,110(14)	65,72 (1)	-	*mut8*	*mut9*
10A			78,57 (39)	91,83 (1)	-	*mut8*	*mut9*
B			131,101(5)	108,107(0)	-	*mut8*	*mut9*
C			119,95 (43)	18,19 (0)	+	*mut8*	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE 120: Presumptive *lys1-1* locus (red) revertants arising on limiting lysine in haploid strains segregating for *mut9* *mut7* or *mut8*

Strain RO507-	Designated *mut*			Red Revertants
1D	7	+		14,18
2D	7	+		25,15
5C	7	+		17,18
7D	+	+		2,0
8A	7	+		16,10
9A	+	9		2,0
11C	7	+		13,10
D	7	+		17,19
RO109-				
1B	+	9		3,4
2A	+	9		4,8
3D	+	9		3,3
4A	+	9		3,0
8C	8	+		5,4
10C	8	+		1,0
11A	8	+		1,0
B	8	+		2,1

Strain RO508-	Designated *mut*			Red Revertants
1B	+	8	9	2,2
D	7	8	+	63,65
2A	7	8	+	68,80
B	+	8	9	6,9
3A	7	+	+	15,16
B	+	+	+	0,2
D	+	8	9	4,2
4A	+	8	9	3,2
B	+	+	+	2,0
D	7	+	+	14,14
5A	+	+	9	2,3
B	7	+	+	7,14
D	+	+	+	0,2
6A	7	+	+	5,13
B	+	+	+	1,2
C	+	8	9	1,2
D	7	8	9	4,4
7A	+	8	+	1,3
B	+	+	+	1,0
8B	+	8	9	2,3
C	7	+	+	11,11
9C	+	+	+	1,0
10C	+	8	+	3,3

TABLE 121 a: Summary of haploid Lassie scores from crosses RO507 and RO109

his1-7 reversion	*MUT*$^+$	*mut9*	Mutator(s)† *mut7*	*mut8*	*mut7mut9*	*mut8mut9*
Mean	14.5	81	59	135	33*	155*
S.E.	1.2	2.8	3.4	9.4	8	30
lys1-1 reversion						
Mean	15.2	145	52	23	5*	102*
	0.9	4.3	2.3	0.7	1	8

* Only two determinations
† Only the allele *mut8-1* was segregating in these crosses

b: Mutation rates (M) from crosses RO89, RO507, RO508, RO109 and RO514 in terms of (unreverted) cells per Lassie test plate

Mutator	RO strain	*his1* allele	Lysine Lassie score*	Cells/ plug* x 10^{-4}	M_{lys} x 10^8	Histidine Lassie score*	Cells/ plug* x 10^{-4}	M_{his} x 10^8
mut9	109-1A	1-7	54	122§	19	100	120	35
	-6C	"	88	132	28	76	116	27
	507-2A	1-1	54(21)	132	17	1	97	0.4
	89-2D	"	57(5)	122§	20	0	91	< 0.5
mut7	507-2D	1-1	41(16)	142	12	1	92	0.5
mut8	109-6D	1-7	34(4)	174	8.2	133	163	34
		1-1	19(5)	139	5.7	3	112	1.1
mut9mut7	507-2B	1-7	21†	89§	9.9	47	68§	29
	508-1C	1-1	58(8)	120§	20		51§	
mut9mut8	109-6A	1-1	63	133§	20	4	103	1.6
MUT$^+$	507-2C	1-7	16	191	3.5	23	112§	8.6
	508-1A	1-1	13(3)	114	4.8	0	128	< 0.3
mut9mut8-2	514-2A	1-7	20(3)	254§	3.3	109	155	29
**	508-2B	1-1	72(5)	139§	22	3	94§	1.3

* Average of two determinations
§ Average of four determinations
† Counted after 14 days
** *mut8-1* is designated '*mut8*', unless otherwise indicated

strain from the first cross produced mostly viable *mut7 mut9* spores. It is therefore possible that background genotype was affecting viability in these crosses, as was the case for crosses of *mut7* to *mut5*.

From the data shown in Tables 115, 116 and 117, *mut7* appears to be unlinked to *mut9* (1P:17T:6N). The *mut9-1* allele does not appear to confer an enhanced *his1-1* reversion frequency.

Table 18 shows the tetrad analysis of a cross of the *mut9* strain RO89-2D to a *mut8-1* strain. The two loci are linked (30P:10T:1N, when the *mut8-2-mut9* recombinant tetrads from Tables 115 and 117 are included in the estimate of linkage), separated by about 20 map units. Table 119 shows the failure of *mut8* to segregate in a cross of a known *mut8-1* strain to a suspect *mut8-2 mut9* strain.

Tables 121a and b summarize the Lassie scores and mutation rates estimated for *mut9* strains uncontaminated with *mut8-2*. As with the *mut7 mut5* strains, *mut7 mut9* haploids appear to be less than additively enhanced for the expression of revertants per cell. The same appears to be true of *mut8 mut9* strains (which resemble *mut8 mut5* haploids).

h. *rad52* (Tables 122, 123 and 124)

A cross of *mut7* to *rad52* was made to determine whether the lethality observed in *mut7 rad51* strains would be seen in *mut7 rad52* strains. The presence of *rad52* in spore clones was detected by their sensitivity to γ -irradiation. Table 122 indicates that

rad52 mut7 was observed to be a lethal combination at 26°C. Two further crosses of *mut7* to *rad52* (Table 4) produced no viable double mutant spore clones in a total of 33 tetrads tested. However, tetrads from the first cross germinated at 15°C yielded *ts*, γ-sensitive spore clones (Table 123). Unfortunately, the low temperature inhibits growth in many strains. Successful exploitation of this result would require further outcrossing of the pertinent mutators into backgrounds amenable to growth at this temperature. Hence, germination of strains heterozygous for *mut5* and *mut7* was not attempted at 15°C.

Neither *mut7* nor *mut8* appears to be closely linked to *rad52* (2P:12T:3N and 0P:9T:0N, respectively), from the data shown in Tables 122, 123 and 124. The *rad52-1* allele does not appear to enhance *his1-1* reversion. It normally confers a six-fold increase in Lassie scores (see also Appendix Table A1), over MUT^+ strains for *his1-7* and *lys1-1* revertant frequency. A *mut7 rad52* strain appeared to be reduced for both *lys1-1* and *his1-7* revertant frequencies when compared with those of either single mutant (Table 123). Additivity could not be excluded for reversion scores at either *lys1-1* or *his1-7* in *mut8 rad52* strains, because rate studies were not performed.

TABLE 122: Phenotypes of spores recovered from cross RO94 ($\frac{mut7}{+}$ $\frac{+}{rad52}$)

Strain RO94-	Segregating alleles at *hom3*	*his1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	after γ	Designated mutator(s)	
1A	+	1-1	0,0 (0)	15,9 (0)	+	+	+	+
B	-	1-7	31,54 (2)	52,54(0)	-	+	*mut7*	+
C	-	1-7	32,27 (2)	59,48 (9)	+	-	+	*rad52*
2A	-	1-7	41,31 (1)	45,46 (2)	+	-	+	*rad52*
B	+	1-1	2,1 (0)	35,28 (0)	-	+	*mut7*	+
C	-	1-7	12,15 (0)	15,20 (0)	+	+	+	+
3A	-	1-7	63,50 (3)	57,52 (4)	+	-	+	*rad52*
B	+	1-1	1,0 (0)	25,18 (0)	+	+	+	+
C	+	1-1	2,1 (0)	35,42 (0)	-	+	*mut7*	+
4A	+	1-1	0,0 (0)	11,11 (0)	+	+	+	+
B	-	1-7	17,17 (1)	10,9 (0)	+	+	+	+
5A	-	1-7	28,30 (0)	37,61 (10)	+	-	+	*rad52*
B	-	1-7	38,41 (1)	35,21 (0)	-	+	*mut7*	+
C	+	1-1	0,0 (0)	7,10 (0)	+	+	+	+
6A	+	1-1	0,0 (0)	20,29 (0)	-	+	*mut7*	+
B	-	1-1	3,2 (0)	45,54 (0)	+	-	+	*rad52*
C	+	1-7	11,20 (0)	11,14 (0)	+	+	+	+
7A	+	1-1	0,0 (0)	5,6 (0)	+	+	+	+
B	-	1-7	17,25 (1)	31,19 (0)	-	+	*mut7*	+
C	-	1-7	25,55 (81)	56,61 (0)	+	-	+	*rad52*
8A	-	1-7	29,40 (1)	27,30 (0)	-	+	*mut7*	+
B	+	1-1	0,1 (0)	57,43 (1)	+	-	+	*rad52*
C	-	1-7	9,14 (0)	17,14 (0)	+	+	+	+
9A	+	1-1	0,0 (0)	33,35 (0)	-	+	*mut7*	+
B	-	1-7	31,35 (36)	30,44 (1)	+	-	+	*rad52*
C	-	1-7	40,27 (2)	22,29 (0)	-	+	*mut7*	+
D	+	1-1	3,1 (0)	51,44 (2)	+	-	+	*rad52*
10A	-	1-7	8,12 (0)	7,22 (0)	+	+	+	+
B	+	1-1	0,0 (0)	28,22 (0)	-	+	*mut7*	+
C	-	1-7	41,42 (0)	47,52 (7)	+	-	+	*rad52*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE 123: Phenotypes of spores recovered from cross RO94($\frac{mut7}{+}$ $\frac{+}{rad52}$) at 15°C.

Strain RO94-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine at 26°C	Prototrophs arising** on limiting histidine at 15°C*	Prototrophs arising** on limiting lysine at 26°C	Prototrophs arising** on limiting lysine at 15°C*	Survival at 36°C	Survival after γ	Designated mutator(s)	
11A	+	1-1	0	0(0)	35	18(0)	-	+	*mut7*	+
B$^{\rho}$	+	1-1	2	1(0)	11	3(0)	+	+	+	+
C$^{\rho}$	-	1-7	117	62(1)	83	69(0)	+	-	+	*rad52*
12A	+	1-1	1	1(0)	17	3(0)	+	+	+	+
B	-	1-7	122	26(0)	103	16(2)	+	-	+	*rad52*
C	+	1-1	0	1(0)	45	12(0)	-	+	*mut7*	+
13A$^{\rho}$	+	1-1	2	0(0)	122	59(1)	+	-	+	*rad52*
B$^{\rho}$	-	1-7	94	112(0)	15	5 (0)	+	+	+	+
C$^{\rho}$	-	1-7	80	6(0)	33	0(0)	-	+	*mut7*	+
14A$^{\rho}$	-	1-7	110	(0)	17	(0)	+	+	+	+
B$^{\rho}$			0	1(0)	1	3(0)	-	-	*mut7*	*rad52*
C	+	1-1	0	0(0)	11	2(0)	+	+	+	+
15A$^{\rho}$			no growth		no growth		-	-	*mut7*	*rad52*
B$^{\rho}$	-	1-7	95	9(0)	6	5(0)	+	+	+	+
C	-	1-7	100	80(0)	13	11(0)	+	+	+	+
16A	-	1-7	109	41(1)	94	18(0)	+	-	+	*rad52*
B	-	1-7	70	(0)	28	21(0)	-	+	*mut7*	+
C	+	1-1	0	0(0)	61	(4)	+	-	+	*rad52*
17A	+	1-1	0	1(0)	9	5(0)	+	+	+	+
B$^{\rho}$	-	1-7	68	36(0)	67	21(0)	+	-	+	*rad52*
C	-	1-7	56	48(3)	30	24(0)	-	+	*mut7*	+
18A	-	1-7	61	11(0)	21	11(1)	-	+	*mut7*	+
B	+	1-1	4	2(0)	69	18(0)	+	-	+	*rad52*
C$^{\rho}$	+	1-1	0	2(0)	44	16(0)	+/-	+/-		
D	-	1-7	95	26(0)	58	20(0)	+	+	+	+

** Numbers of prototrophs existing (at 15°C) prior to growth on limiting medium are parenthesized.

ρ These strains fail to grow when replica plated to YG medium.

* Lassie plates were incubated for ten days instead of the usual six.

TABLE 124: Phenotypes of spores recovered from cross RO111 ($\frac{mut8}{+}$ $\frac{+}{rad52}$)

Strain RO111-	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine	Prototrophs arising** on limiting lysine	Growth after γ	Designated mutator(s)
1A	+	1-1	2,2 (1)	10,21 (0)	+	*mut8* +
B	-	1-7	16,17 (1)	7,11 (0)	+	+ +
C	-	1-7	239,198(18)	91,103(1)	-	*mut8* *rad52*
D	+	1-1	2,0 (0)	68,88 (3)	-	+ *rad52*
2A	+	1-1	3,1 (0)	18,21 (0)	+	*mut8* +
B	-	1-7	65,43 (0)	81,79 (0)	-	+ *rad52*
C	+	1-1	0,0 (0)	8,17 (0)	+	+ +
D	-	1-7	217,279(26)	87,85 (0)	-	*mut8* *rad52*
3A	-	1-7	160,134(18)	72,78 (1)	-	*rad52*
B	+	1-1	4,6 (0)	15,22 (0)	+	*mut8* +
C	+	1-7	44,29 (3)	111,78 (1)	-	+ *rad52*
D	-	1-1	1,0 (0)	18,12 (0)	+	+ +
4A	-	1-7	106,145(54)	20,16 (0)	+	*mut8* +
B	-	1-7	164,186(13)	110,81 (0)	-	*mut8* *rad52*
C	+	1-1	0,1 (0)	11,5 (0)	+	+ +
D	+	1-1	0,1 (0)	80,75 (7)	-	+ *rad52*
5A	+	1-1	0,0 (0)	16,15 (0)	+	+ +
B	+	1-1	0,2 (0)	56,81 (0)	-	+ *rad52*
C	-	1-7	132,175(50)	71,46 (0)	-	*mut8* *rad52*
D	-	1-7	129,159(35)	11,16 (0)	+	*mut8* +
6A	+	1-1	5,0 (0)	21,24 (0)	+	*mut8* +
B	+	1-1	1,0 (0)	24,22 (3)	+	+ +
C	-	1-7	192,210(20)	66,65 (0)	-	*mut8* *rad52*
D	-	1-7	40,63 (5)	82,82 (0)	-	+ *rad52*
7A	+	1-1	0,0 (0)	9,21 (0)	+	+ +
B	-	1-7	179,165(14)	72,70 (0)	-	*mut8* *rad52*
C	+	1-1	1,1 (0)	68,73 (0)	-	+ *rad52*
D	-	1-7	143,119(31)	22,26 (0)	+	*mut8* +
8A	+	1-7	176,168(18)	69,99 (0)	-	*mut8* *rad52*
B	+	1-1	2,0 (0)	46,86 (0)	-	+ *rad52*
C	-	1-1	2,1 (0)	11,11 (0)	+	*mut8* +
D	-	1-7	16,14 (0)	6,13 (0)	+	+ +
9A	+	1-1	3,3 (0)	21,18 (0)	+	*mut8* +
B	+	1-1	1,0 (0)	14,18 (0)	+	+ +
C	-	1-7	40,42 (4)	65,57 (0)	-	+ *rad52*
D	-	1-7	144,117(22)	22,15 (0)	+	*mut8* +
10A	+	1-1	1,0 (0)	69,60 (0)	-	*rad52*
B	-	1-7	53,38 (1)	58,56 (0)	-	+ *rad52*
C	-	1-7	104,129(36)	29,19 (0)	+	*mut8* +
D	+	1-1	1,0 (0)	10,11 (0)	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

DISCUSSION

A. Phenotypes of the *mut7-1* and *mut8-1* Alleles.

This study has shown that a functional *MUT7* gene is required for cell viability. The partially functional allele *mut7-1* confers *cdc* and mutator phenotypes, enhanced recombination at *his1*, and MMS sensitivity, as well as conditional inviability when combined with *mut5*. These phenotypes all corevert with *mut7*-mediated temperature sensitivity. This allele is probably also responsible for the cessation of net DNA synthesis in a *mut7* strain incubated at 36°C. The data from one cross suggests that *mut7* may be loosely linked to *lys1*.

The *mut8-1* mutation is as enigmatic at the end of this study as it was at the beginning. Although it confers no known sensitivities to mutagens, it interacts with *mut7*, which does have altered repair capacities. The result of this interaction (in *mut7 mut8* strains) is an approximate twenty-fold increase in mutation rate for new auxotrophs, and for *lys1-1*, *hom3-10* and *his1-1* revertant frequency, over MUT^+ levels. Suppressor revertants of *lys1-1* are only enhanced four-fold, whereas *his1-7* revertants are enhanced fifty-fold. The *mut8* locus is linked to *mut9*.

An Upper Limit to Spontaneous Mutation?

There is some indication that if mutation rates were in excess of those observed in *mut7 mut8* strains the result might be an inviable strain of yeast. Based on a twenty-fold increase in mutation rate per base pair per cell from 10^{-8}, it may be calculated that a

significant proportion of yeast cells will die due to mutations occurring in essential genes. This expectation may have been observed as a reduction of plating efficiency in *mut7 mut8* strains. The highest levels of *induced* mutation frequency rarely exceed 10^{-1} per surviving cell (compared to 10^{-2} in *mut7 mut8* strains) for auxotroph induction in yeast (Lindegren, 1965) or 10^{-3} for reversion at a single locus in yeast (see for example, Morrison 1978). This may be due to the occurrence of numbers of forward mutations similar to those occurring in *mut7 mut8* strains. In the case of *lys1-1* reversion there are more than ten loci affecting the metabolic pathway to lysine which may be inactivated by mutation, even as a *lys1-1* allele is (UV-) reverted. As reversion rates per locus approach 10^{-2}, the forward mutation rate to lysine auxotrophy at *lys1* and other loci may approach 1. As this state is approached, the number of revertants *per survivor* should achieve a maximum and then rapidly decrease.

B. The "*mut8* Effect"

The presence of *mut8* is correlated with a stationary-phase enhancement of reversion for at least one test allele, *his1-7*. It is not known whether this enhancement is due to cell arrest, or whether the *mut8* gene product is expressed in the G1 stage of the cell cycle. It was assumed that the "*mut8* effect" was responsible for the enhanced numbers of revertants appearing in *mut8* strains on the -his plates used to detect pre-existing histidine prototrophs in Lassie tests. But it is probable that this phenotype is not due to *mut8*

alone. The effect was often reduced in *mut8* strains segregating from crosses involving *mut7 mut8* and *mut5*. This was particularly evident in viable *mut7 mut8 mut5* haploids.

C. *mut7* as a *cdc* Mutation

The G1, *cdc* phenotype seen in *mut7* cells suggests a trivial interpretation of the *mut7 mut8*-mediated, synergistic enhancement of spontaneous mutation. If *mut8* confers enhanced G1 mutability, and if *mut7* causes cells to spend more time in G1 at 26°C, much more *mut8*-mediated mutation might occur in the double mutator mutant.

The observation that net DNA synthesis was halted in a *mut7* strain held at 36°C seems to contradict the results of von Borstel and Johnston (unpublished data), which indicated that uptake of label into DNA persisted at a reduced rate for several hours following the shift to 36°C. These data are not incompatible if it is assumed that residual uptake is due to mitochondrial DNA synthesis (in the uptake experiment) or to turnover of DNA without net synthesis.

D. The *his1-1* allele and Intragenic Recombination at *his1*

The genetic studies of *mut7* resulted in the extensive analysis of two new Lassie test systems. The allele *his1-1*, considered to be a frameshift mutation by Magni (1963), was found to have very low spontaneous reversion frequencies compared to *his1-7*. Low histidine Lassie scores were observed in all *his1-1* strains, except those bearing *mut8*. This phenotype, together with the close linkage of *hom3* to *his1* (*his1-7* in most crosses) permitted the positive identification

of a *his1* allele without the need for further allelism testing. The difference in Lassie test scores between the two alleles was also seen in diploids. In addition, intragenic recombination between the two alleles was easily observed as a five to ten-fold increase in histidine Lassie scores relative to the (*his1-7*) reversion frequency. The recombination test system could be improved by the introduction of *arg6* as a flanking marker, and by the use of a *his1-7 his1-1* double mutant control. Note that the system would be adaptable to studies of spontaneous recombination at *arg4*, *his5* or *lys1*, since all of these loci may be Lassie-tested.

A study of *HIS1*$^+$ mitotic recombinants arising in the Lassie test revealed that these probably arose by the preferential conversion of *his1-1*, in a *MUT*$^+$ homozygote. This specificity was reduced in a related *mut7/mut7* diploid.

The above interpretation must be qualified by the following information. Crossfeeding revertants did not show conversion at *his1-1*. The apparent conversion of *his1-7* was noted in six crossfeeding prototrophs tested. This, together with the observation that certain *MUT*$^+$ diploid strains produced no crossfeeders on Lassie test plates leads to several speculations. Either these strains are totally specific for *his1-1* conversion, or the conversion of *his1-7* to + normally transfers an auxotrophic, second-site, crossfeeding allele to the converted strand. This allele may be missing in the diploids which did not have crossfeeder revertants. A third possible explanation is that some crossfeeders were dominant for *his1* prototrophy but often recessive for crossfeeding ability (Fogel *et al.*, 1978).

However, no crossfeeders were observed in any haploids derived from the sporulated *HIS*$^+$ recombinants, other than those from crossfeeding prototrophs. (The suggestion which predicts that some strains carry a pre-existing, crossfeeding mutation in the *his1* gene would explain why roughly 80% of the *his1-1* revertants observed in the study of Fogel *et al.* (1978) and in this study were crossfeeders. However, this interpretation requires that all non-feeder, *HIS1-7*$^+$ prototrophs which arose in *mut7* homozygotes were not converted, but reverted, or else not co-converted for the crossfeeding allele. While the above possibility exists it does not explain why certain *MUT*$^+$, *his1-7* homozygotes or *MUT*$^+$/*MUT*$^+$, *his1*-heteroallelic crosses had *no* feeder revertants.)

When other *mut* strains were examined for spontaneous intragenic recombination at *his1*, only those bearing *mut5* were consistently observed to be different from *MUT*$^+$ controls. A homozygous *mut5* diploid was reduced for recombination, and several heterozygous *mut5* strains also appeared to be reduced. The other γ -radition sensitive locus tested, *mut9*, did not appear to affect *his1* recombination, but this result remains to be confirmed in a genetic background free of *mut8-2*. The latter allele must have been present in the original *mut9* strain used in this study, XV396-1A. (It had been assumed that *mut7* and *mut8* were different from all other *mut* loci, and the evidence from diploid complementation tests confirmed this belief, with the single "exception" of *mut8-2*.)

E. Dominance of *mut5* and *mut6*

Two observations were made concerning the dominance of *mut5* and *mut6* for different repair parameters. The *mut5* (*rad51*) mutation appeared to be dominant with respect to MMS sensitivity. All *mut6* heterozygotes studied were recessive for the spontaneous reversion of *his1-7/his1-7*, except when *mut8* was homozygous in the same diploid. The latter observation is discussed in the following section.

F. Interactions of Double Mutator Mutants

Tetrad analysis of $\frac{mut7}{+}\ \frac{+}{mut}$ and of $\frac{mut8}{+}\ \frac{+}{mut}$ strains bearing *mut1*, *mut2*, *mut3* or *mut4* has indicated that additivity of separate mutator activities could not be ruled out when accounting for double mutator mutant mutator activities (e.g. *mut7 mut3*). The exceptions were *mut1 mut7*-conferred mutator activity for lysine reversion, which was synergistically enhanced, and *mut4 mut7* and *mut4 mut8*-conferred mutator activity at *his1-7*, which were both less than additively enhanced. It should be mentioned that *mut8* is the only mutator locus tested not to have interacted synergistically with *mut1* for *lys1-1* mutator activity.

No viable *mut5 mut7* segregants were recovered from tetrads of $\frac{mut7}{+}\ \frac{+}{mut5}$ diploids in one genetic background. In another background, about one half of the expected *mut7 mut5* spores were viable, suggesting that another genetic factor was responsible for the (in)viability, in addition to *mut7* and *mut5*. Mutator activity was reduced in viable *mut7 mut5* spore clones. The "*mut8* effect" was also reduced in *mut7 mut8 mut5* strains, as evidenced by low numbers of pre-existing *his1-7* revertants observed in Lassie tests of those strains.

Strains bearing *mut8 mut5* appeared to be less than additively enhanced for mutator activity at both *his1-7* and *lys1-1*. However, rather than one mutator being epistatic to the other, it seems that there is a mutual exclusiveness of expressed mutations. It would appear that only *mut8* effected mutation at *his1-7*, while only *mut5* effected *lys1-1* (total) reversion in the double mutator mutants. If true, this may mean that the *mut8* and *mut5* mutations cause these loci to mutate by mutually exclusive, but possibly competing mechanisms. Such an interpretation of epistasis may be expanded to account for the more complex situation observed in crosses involving *mut6*.

Double mutant *mut7 mut6* haploids express both *his1-7* and *lys1-1* reversion as if the latter were *mut6*-mediated. In addition, the numbers of *lys1-1* locus revertants normally observed in *mut7* strains were not observed in an NPD-derived *mut7 mut6* strain. The "pathway" interpretation of *mut6* epistasis for mutability would predict that these two mutants block the same repair pathway, on the basis of their epistatic relationship. In addition, they should confer a similar synergistic effect upon a third mutator mutation in a different repair pathway competing for similar lesions. (Another prediction might be made, based on the *mut7 mut6* epistatic relationship. If repair pathway relationships hold for lethal interactions, *mut6* may also be lethal in combination with *mut5*.)

The first prediction is partly borne out, in that *mut6 mut8* confers a synergistic enhancement of *his1-7* reversion. However, for *lys1-1* reversion epistasis appeared again.

If mutual exclusiveness were invoked to account for this dichotomy, one might propose that *MUT6*$^+$ repair has no access to lesions which could mutate the *lys1-1* allele, and that the same is true of *MUT7*$^+$ repair and the suppressor loci. Reversion of *his1-7* might be accomplished by either allele's gene product, but one should exclude the other, to account for *mut6 mut8* and *mut7 mut8* synergistic enhancement of mutator activity at *his1-7*. Such inaccessibility may be due either to cell cycle or enzyme specifities (for different substrates in the latter suggestion).

The appearance of *mut6/+* dominance for *his1-7/his1-7* reversion in a *mut8/mut8* background may indicate that *mut8* effects a type of lesion which repair processes resolve mutagenically. This might be true whether or not the *MUT8*$^+$ gene product normally contributes to repair. If true, such *mut8*-mediated lesions are probably not responsible for most spontaneous mutation at *his1-7* in *MUT*$^+$ strains, because of the normal recessiveness shown by *mut6/+* re mutator activity at *his1-7*. One further implication of the proposal would be that *mut8* does not confer enhanced production of lesions at suppressor loci.

Finally, tetrad analyses of crosses of the alleles *mut5-1* (*rad51*), *mut9-1* and *rad 52-1* to *mut7-1* indicate that the γ -radiation-sensitive phenotype is generally associated with reduced viability in strains also bearing *mut7*.

G. Possible Reasons for *mut7 mut5* Mediated Cell Death.

From the perspective of repair mechanisms in yeast, it is possible to construct models which may explain the *mut7 mut5* interaction. A trivial explanation would be that *mut7* and *mut5* each block one of

two pathways for the repair of some common spontaneous lesion, whose presence would cause cell death if unresolved. Other pathways for the repair of this lesion would have to exist, however, or else *mut7* or *mut5* are leaky in some backgrounds. The presence of viable *mut7 mut5* segregants renders insufficient any simple explanation based on known or implied repair pathways. This is mainly because the latter cannot take cell cycle-mediated specificities, or overlapping enzyme specificities for repair into account at this time.

Another plausible explanation of the *mut7 mut5*-mediated inviability is that the presence of *mut7* results in neighboring lesions being induced on both strands of a DNA duplex. In *mut5* strains such overlapping lesions may not be resolved. Mowat (1979) and Resnick and Martin (1976) noted that two-strand breaks are not repaired in *rad51* and *rad52* yeast strains, respectively. Cross-linking agents are excised in a *rad51* strain but this is followed by the inability to repair the double strand lesion induced (Jachymczyk, personal communication).

The failure of *mut5* homozygotes to recombine at *his1* spontaneously (this thesis) or following UV-irradiation (Morrison, 1979) suggests that spontaneous double-strand lesions caused ultimately by base mispairs (at least in the case of MMS) must have functional recombination repair to be successfully resolved.

Since *mut7* confers enhanced recombination in diploids, it may also provoke G2 chromatid exchange in haploids. Haploid *mut5* strains are sensitive to γ-irradiation (Quah, unpublished data). Hence, the diploid phenotypes of both these loci may be acting in haploid spores to produce the lethal interaction.

In bacteria, several inviable combinations of genes associated with repair or replication have been noted; *recA polA* (Gross *et al.*, 1971), *recB polA* (Monk and Kinross, 1972) *recA dam3* (Marinus and Morris, 1974), and *polA lig4* (Gottesman *et al.*, 1973). These indicate the interrelatedness of replication and repair processes in cell function. The inability of *recA* cells to resolve nicks or gaps in DNA caused by the presence of *dam3* or *polA* is the probable cause of the lethality in *recA polA* or *recA dam3* strains, while the *polA lig4*-conferred lethality was thought to be due to lesions created during DNA replication.

A comparison of the *mut7 mut5* interaction with the above indicates that *mut7-1* is not a *recA* or *recB* type allele because *mut7-1* strains are not sensitive to either UV or γ -irradiation. Nor is *mut7-1* likely to be a ligase mutant, since it complements *cdc9*, the only known *lig* mutant in yeast, for growth at 36°C (von Borstel, pers. comm.). Louise Prakash (pers. comm.) has not detected altered polymerase levels or activity, compared to controls, in the *mut7* strain RO428-6C. Hence the only remaining bacterial locus of the several noted above which may resemble *mut7* is *dam3*. However, one must bear in mind the possible occurrence of lethal combinations other than those described here.

The postulated inability of *mut7* strains to recognise which strand to repair is supported by the *mut7* phenotype, which is reminiscent of both ethionine-mediated lethality, and of the *dam3* phenotype, in *E. coli*. Ethionine inhibition of DNA methylation leads to arrested cell division because of the restriction of unmethylated duplex DNA. (Lark, 1968). The resemblance of *mut7* to a *dam* mutation is noteworthy.

Both enhance spontaneous recombination and mutation, are MMS sensitive and cause lethality in a recombination defective background (*dam* mutant pleiotrophies were noted in Marinus and Morris, 1974).

The high-temperature induced lethality observed in *mut7* strains might be explained as an absolute failure to modify daughter DNA half-strands following replication. The apparent failure of a *mut7* strain to increase net DNA when incubated at 36°C, while still incorporating label, could then be rationalized as due to degradation of unmodified DNA. Reduced modification of daughter strands in *mut7* strains incubated at 26°C might prevent a *mut7* cell from recognising and using the parental template for the correct excision of base mispairs. Such excision might be induced during replication, or excision repair. Instead, lack of strand recognition would lead to possibly overlapping excision repair on both strands, and therefore to the mutagenic resolution of mispairs, to double strand lesions (if not breaks) and to recombination. Based upon these speculations, one might predict that *mut7* would be sensitive to base analogues, further enhanced for *his1* recombination at 36°C, and altered for DNA modification, particularly at 36°C. (Yeast have recently been shown to contain 5-methyl-cytosine; Hattman, 1978).

One interpretation of the altered specificity shown by *mut7* strains during spontaneous *his1* intragenic recombination is that this mutator is no longer able to recognise a strand-specific marker effect in heteroduplex DNA. As a result, the spontaneous repair of neighboring mispairs might be occurring on opposite strands of DNA. The double stranded lesion created would mimic MMS damage in that both would then require the *MUT5*$^{+}$ mediated repair mechanism. The repair of

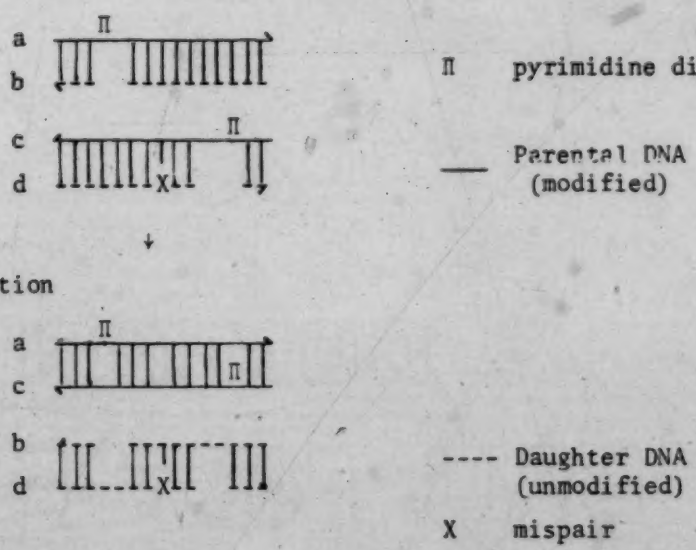
MMS-induced double strand breaks has been shown to require the presence of a duplicate genome in haploid yeast (Chlebowicz and Jachymczyk, 1979).

The total failure to repair base mispairs in DNA could lead to the eventual segregation of mutated duplexes. James and Kilbey (1978) observed that only 2% of all viable colonies tested had recessive lethal mutations expressed in G1, when *rad1/rad1* excision defective, diploid, stationary phase yeast were U.V. irradiated to 90% survival. 24% of the survivors had these mutations expressed in G2, while 25% did not arise until after the G1 of the following generation, or subsequent generations. It would seem that the failure to remove pyrimidine dimers in *rad1* homozygotes (see Unrau, 1971; Reynolds, 1979) allows that lesion to persist through replication (see Kilbey and James, 1979) and also seems to allow the enhanced segregation of mutations. A previous study (James and Kilbey, 1977) had shown that 17% of viable RAD^+/RAD^+ strains had recessive lethal mutations expressed in G1 when similarly irradiated. Only 8% of the survivors had mutations expressed in G2, and *no* mutations were observed to be expressed in subsequent generations.

Lawrence and Christensen (1978) noted that at least one site of U.V.-induced mutation in the *cyc1* locus was not a potential pyrimidine dimer site. Based on the implied existance of strand specificity in the repair of some types of damaged DNA, both of the above results may be explained, as follows.

Mutations might be occurring in UV-irradiated *rad1* homozygotes which recombined to resolve daughter strand gaps (Resnick 1976),

following DNA synthesis. Two potential results of this would be the generation of a two strand lesion (amenable to *RAD51*$^{+}$ repair), and an unbroken, dimer containing strand, as may be seen in the following diagram.



The former might produce the enhanced UV-induced recombination observed in *rad1* strains, the latter would result in the continuing segregation of dimer-containing DNA. If daughter strand base mispairs existed in unmodified DNA (duplex b-d) the cell would not be able to recognize which base pair to excise, and so would produce an increased number of mutations. Furthermore, it is possible that mispairs arising near dimers would not be resolved correctly even prior to recombination since the parental (dimer-containing) strand might not be available for use as a template.

The mutations expressed in G1 in *RAD*$^{+}$ homozygotes might be due to mispairs created during the excision-repair of dimers on

modified strands of DNA, and opposite unmodified strands. The cell would have no modified template for comparison, and so would frequently misrepair the mispair.

If the inability to repair base mispairs correctly is responsible for *mut7 mut5* lethality, how can the viability of some *mut7 mut5* strains be explained? A defect in the excision repair of the types of damage to DNA that normally led to *mut7* mediated misrepair might be expected to reduce *mut7 mut5*-mediated inviability. It has recently been observed (von Borstel, pers. comm.) that a deficiency in the liquid holding recovery of U.V. damage is segregating in strains related to those used in this study. Interestingly enough, Strike and Emmerson (1972) found that *polA recB* strains of *E. coli* (normally inviable) could be isolated in the presence of the *sbcA*$^-$ (exonuclease deficient; Kushner *et al.*, 1972) mutation.

Whether or not *mut7* is shown to confer reduced strand-specificity of repair, mispairs arising during DNA replication or repair are a possible major source of spontaneous lesions. If it can be ascertained whether yeast possess a DNA modification (or other strand recognisant) apparatus for the purpose of editing this type of damage, one will be closer to understanding the origins of its spontaneous mutation.

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APPENDICES

TABLE A1: Phenotypes of spores recovered from cross RO61(*mut1/+*); and of spores capable of growth at (a) 36°C from cross RO71(*mut1/+*)

Strain RO61-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	33	(0)	7	(0)	+
B	144	(5)	337	(0)	*mut1*
C	52	(0)	80	(61)*	+
D	243	(2)	397	(7)	*mut1*
2A	32	(1)	17	(0)	+
B	142	(6)	178	(41)	*mut1*
C	127	(7)	275	(12)	*mut1*
D	28	(0)	17	(0)	+
3A	207	(0)	381	(5)	*mut1*
B	45	(0)	21	(0)	+
C	108	(2)	192	(258)	*mut1*
D	32	(0)	9	(0)	+
4A	93	(0)	240	(31)	*mut1*
B	23	(0)	20	(35)	+
C	10	(2)	20	(0)	+
D	57	(3)	269	(3)	*mut1*
†5A	121	(18)	409	(10)	*mut1*
B	98	(6)	324	(2)	*mut1*
C	29	(53)	176	(6)	*mut1*
D	19	(0)	17	(0)	+

Strain RO71-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	27	(0)	13	(0)	+
D	58	(1)	219	(0)	*mut1*
2B	27	(3)	6	(0)	+
D	95	(3)	373	(2)	*mut1*
3B	18	(2)	7	(0)	+
D	17	(1)	20	(0)	+
4A	32	(1)	15	(0)	+
D	25	(0)	12	(0)	+
5C	35	(0)	226	(2)	*mut1*
D	51	(2)	299	(2)	*mut1*
6B	72	(0)	349	(1)	*mut1*
D	20	(0)	9	(0)	+
7A	14	(1)	14	(0)	+
C	58	(1)	334	(1)	*mut1*
8C	53	(1)	336	(5)	*mut1*
D	17	(0)	18	(0)	+
9B	25	(1)	27	(0)	+
D	27	(1)	10	(0)	+
10B	28	(0)	4	(0)	+
C	18	(0)	17	(0)	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

δ Only *his 1-7* is present in these crosses. † False tetrad.

TABLE A1: Phenotypes of spores recovered from cross RO62(*mut2/+*); and of spores capable of growth at (b) 36°C from cross RO72(*mut2/+*)

Strain RO62-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	98	(13)	106	(2)	*mut2*
B	11	(0)	16	(0)	+
C	72	(4)	88	(5)	*mut2*
D	18	(0)	6	(1)	+
2A	95	(4)	95	(0)	*mut2*
B	17	(3)	5	(12)	+
C	53	(2)	59	(3)	*mut2*
D	12	(0)	11	(0)	+
3A	79	(5)	62	(129)	*mut2*
B	14	(1)	15	(0)	+
C	76	(8)	78	(64)	*mut2*
D	11	(0)	9	(0)	+
4A	17	(0)	11	(0)	+
B	14	(0)	9	(0)	+
C	83	(8)	80	(2)	*mut2*
D	78	(8)	66	(1)	*mut2*
5A	16	(1)	11	(0)	+
B	18	(0)	12	(0)	+
C	60	(1)	70	(0)	*mut2*
D	48	(13)	70	(4)	*mut2*

Strain RO72-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	19	(1)	7	(0)	+
C	82	(1)	104	(1)	*mut2*
2C	13	(0)	15	(0)	+
D	66	(71)	78	(0)	*mut2*
3A	62	(6)	82	(1)	*mut2*
D	8	(0)	7	(0)	+
4B	5	(0)	6	(0)	+
C	70	(0)	130	(10)	*mut2*
5C	40	(0)	12	(0)	+
D	77	(4)	74	(0)	*mut2*
6A	69	(3)	118	(1)	*mut2*
D	93	(16)	84	(1)	*mut2*
7A	65	(0)	118	(2)	*mut2*
D	5	(0)	4	(0)	+
8A	10	(0)	6	(0)	+
C	79	(1)	106	(6)	*mut2*
9A	15	(3)	13	(0)	+
D	86	(0)	56	(3)	*mut2*
10C	13	(0)	8	(0)	+
D	20	(24)	6	(0)	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

δ Only *his1-7* is present in these spores.

TABLE A1: Phenotypes of spores recovered from cross RO63(*mut3/+*); and of spores capable of growth at (c) 36°C from cross RO73(*mut3/+*)

Strain RO63-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	11	(0)	5	(0)	+
B	79	(4)	68	(12)	*mut3*
C	80	(2)	80	(33)	*mut3*
D	9	(0)	14	(1)	+
2A	123	(31)	72	(1)	*mut3*
B	19	(0)	15	(0)	+
C	7	(2)	14	(0)	+
D	70	(26)	73	(0)	*mut3*
3A	83	(10)	67	(1)	*mut3*
B	12	(0)	12	(0)	+
C	5	(1)	16	(0)	+
D	83	(3)	70	(0)	*mut3*
4A	80	(4)	81	(0)	*mut3*
B	11	(0)	7	(0)	+
C	106	(0)	65	(4)	*mut3*
D	8	(1)	7	(0)	+
5A	66	(6)	69	(0)	*mut3*
B	7	(1)	4	(0)	+
C	79	(2)	82	(0)	*mut3*
D	7	(0)	9	(0)	+

Strain RO73-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1A	18	(0)	11	(0)	+
C	17	(0)	6	(0)	+
2A	63	(7)	59	(1)	*mut3*
B	20	(0)	13	(0)	+
3A	14	(0)	13	(0)	+
D	60	(9)	85	(0)	*mut3*
4A	*		58	(0)	*mut3*
D	10	(3)	10	(0)	+
5C	13	(2)	9	(0)	+
D	52	(3)	83	(4)	*mut3*
6B	42	(0)	57	(0)	*mut3*
C	11	(0)	5	(0)	+
7A	81	(10)	181	(1)	*mut3*
C	10	(0)	14	(0)	+
8C	62	(5)	99	(0)	*mut3*
D	9	(0)	10	(0)	+
9A	86	(5)	60	(0)	*mut3*
C	10	(5)	9	(0)	+
10A	39	(100)	53	(0)	*mut3*
C	66	(8)	61	(0)	*mut3*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

δ Only *his1-7* is present in these spores.

* 'Jackpot' not scored.

TABLE A1: Phenotypes of spores recovered from cross RO64(*mut4/+*); and of spores capable of growth at 36°C from cross RO74(*mut4/+*)
(d)

Strain RO64-	Segregating alleles at *hom3*	*his1*	*his5*	Prototrophs arising** on limiting histidine		lysine		Designated mutator
1A	-	1-7	5-2	0	(0)	16	(0)	+
B	+	+	+			9	(0)	+
C	-	1-7	+	11	(0)	5	(0)	+
D	+	+	5-2	62	(32)	115	(22)	*mut4*
2A	-	1-7	+	9	(0)	11	(0)	+
B	+	+	5-2	69	(0)	106	(0)	*mut4*
C	+	+	5-2	8	(0)	12	(0)	+
D	-	1-7	+	47	(1)	25	(0)	*mut4*
3A	+	+	+			100	(0)	*mut4*
B	-	1-7	5-2	0	(0)	8	(0)	+
C	+	+	5-2	11	(0)	26	(2)	+
D	-	1-7	+	66	(0)	50	(2)	*mut4*
4A	+	+	5-2	5	(1)	11	(2)	+
B	-	1-7	5-2	0	(0)	43	(0)	*mut4*
C	+	+	+			36	(3)	*mut4*
D	-	1-7	+	13	(1)	14	(0)	+
5A	-	1-7	+	49	(16)	51	(10)	*mut4*
B	-	1-7	5-2	0	(0)	8	(0)	+
C	+	+	+			12	(0)	+
D	+	+	5-2	31	(0)	59	(0)	*mut4*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

Strain RO74-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1B	113	(10)	52	(0)	*mut4*
D	19	(2)	7	(0)	+
2C	53	(8)	44	(2)	*mut4*
D	37	(2)	12	(0)	+
3A	71	(3)	51	(1)	*mut4*
D	40	(2)	67	(0)	*mut4*
4A	42	(0)	48	(0)	*mut4*
D	89	(3)	50	(0)	*mut4*
5C	33	(0)	12	(0)	+
D	19	(1)	13	(0)	+
6A	9	(1)	5	(0)	+
D	11	(1)	9	(0)	+
7C	83	(2)	99	(0)	*mut4*
D	26	(1)	8	(0)	+
8B	55	(5)	55	(0)	*mut4*
D	12	(1)	8	(0)	+
9A	59	(1)	27	(13)	*mut4*
D	15	(2)	10	(0)	+
10A	85	(0)	51	(1)	*mut4*
B	79	(84)	68	(2)	*mut4*

δ Only *his1-7* is present in these spores.

TABLE A1: Phenotypes of spores recovered from cross RO65(*mut5/+*); and of spores capable of growth at (e) 36°C from cross RO75(*mut5/+*)

Strain RO65-	Segregating alleles at *hom3*	*his1*	*his5*	Prototrophs arising** on limiting histidine		lysine		Designated mutator
1A	-	+	5-2	84	(1)	45	(0)	*mut5*
B	-	1-7	+	41	(6)	14	(0)	+
C	+	1-7	+	40	(0)	7	(0)	+
D	+	+	5-2	99	(6)	29	(5)	*mut5*
2A	-	1-7	5-2	0	(0)	11	(0)	+
B	-	1-7	+	31	(0)	13	(0)	+
C	+	+	5-2	103	(3)	70	(0)	*mut5*
D	+	+	+			17	(4)	*mut5*
3A	-	1-7	+	25	(5)	11	(0)	+
B	+	+	5-2	110	(1)	31	(1)	*mut5*
C	+	+	+			53	(3)	*mut5*
D	-	1-7	5-2	0	(0)	14	(0)	+
4A	-	1-7	5-2	0	(0)	15	(0)	+
B	-	1-7	5-2	0	(0)	41	(20)	*mut5*
C	+	+	+			29	(1)	*mut5*
D	+	+	+			6	(0)	+
5A	-	1-7	+	189	(3)	48	(9)	*mut5*
B	-	1-7	5-2	0	(0)	11	(0)	+
C	+	+	5-2	106	(2)	45	(0)	*mut5*
D	+	+	+			6	(0)	+
6A	+	+	5-2	118	(17)	61	(12)	*mut5*
B	-	1-7	5-2	0	(0)	11	(0)	+
C	-	1-7	+	27	(3)	9	(0)	+
D	+	+	+			42	(0)	*mut5*

All designated RO65 *mut5* strains approximately doubled the above prototroph frequencies after 14 days incubation.

Strain RO75-	Prototrophs arising** on limiting histidine[δ]		lysine		Designated mutator
1B	79	(14)	54	(6)	*mut5*
D	94	(6)	71	(1)	*mut5*
2C	27	(0)	8	(0)	+
D	30	(2)	15	(0)	+
3A	96	(1)	47	(1)	*mut5*
4A	32	(1)	18	(0)	+
B	89	(4)	76	(9)	*mut5*
5A	94	(0)	60	(0)	*mut5*
C	33	(1)	14	(0)	+
6A	19	(2)	5	(0)	+
D	21	(1)	18	(0)	+
7A	70	(1)	50	(3)	*mut5*
B	37	(3)	10	(0)	+
8B	25	(1)	17	(0)	+
C	26	(1)	6	(1)	+
9B	82	(14)	45	(0)	*mut5*
C	14	(0)	19	(0)	+
10A	38	(2)	15	(0)	+
C	81	(0)	70	(1)	*mut5*

δ Only *his1-7* is present in these spores.
** Numbers of prototrophs existing prior to growth on limiting medium are bracketed.

TABLE A1: Phenotypes of spores recovered from cross RO66(*mut6/+*); and of spores capable of growth at 36°C from cross RO76(*mut6/+*), the latter having been pre-grown on YG medium.

Strain RO66-	Prototrophs arising** on limiting histidine[δ]	Prototrophs arising** on limiting lysine	Growth on YG	Designated mutator
1A	15 (1)	17 (0)	-	
B	38 (2)	65 (0)	+	*mut6*
C	12 (1)	13 (0)	+	+
D	7 (0)	9 (0)	+	+
2A	25 (2)	86 (1)	+	*mut6*
B	63 (2)	84 (0)	+	*mut6*
C	15 (0)	11 (2)	-	+
D	6 (2)	8 (0)	+	+
3A	15 (1)	6 (0)	-	
B	27 (2)	16 (0)	+	+
C	13 (0)	14 (1)	+	+
D	14 (2)	17 (0)	-	
4A	18 (1)	19 (0)	+	+
B	27 (2)	22 (0)	-	
C	17 (1)	26 (0)	+	+
D	7 (2)	7 (0)	-	
5A	16 (5)	19 (1)	+/-	
B	6 (1)	15 (0)	+	+
C	42 (0)	54 (2)	+	*mut6*
D	13 (2)	14 (1)	+	+
6A	68 (5)	73 (1)	+	*mut6*
B	37 (3)	58 (0)	+	*mut6*
C	5 (0)	18 (0)	+	+
D	17 (2)	16 (1)	+	+

Strain RO76-	Prototrophs arising** on limiting histidine[δ]	Prototrophs arising** on limiting lysine	Growth on YG	Designated mutator
1A	22,15(1)	20,18 (0)	+	+
C	46,54 (1)	45,53 (0)	+/-	*mut6*
2A			-	
B	15,13 (0)	17,12 (0)	+	+
3C	13,12 (0)	19,15 (1)	+	+
4D	18,18 (1)	17,14 (0)	+	+
5C	26,26 (0)	53,54 (0)	+	*mut6*
D	9,12 (1)	12,9 (0)	+	+
6D	43,52 (1)	79,56 (0)	+/-	*mut6*
7D	28,25 (0)	16,12 (0)	+	+
8A	10,20 (1)	15,6 (0)	+	+
9A	11,16 (1)	12,12 (0)	+	+
D	117,33 (86)	46,53 (2)	+/-	*mut6*
10A	23,15 (0)	21,12 (0)	+	+

** Numbers of prototrophs arising prior to growth on limiting medium are parenthesized.

δ Only *his1-7* is present in these spores.

TABLE A1: 'Lassie' phenotypes of spores from cross RO76 pre-grown (g) on YD medium

Strain RO76-	Prototrophs arising** on limiting histidine[δ]	lysine	Growth on YG	Designated mutator
1A	16,19(1)	18,23 (0)	+	+
C	30,27(5)	32,42 (1)	-	
2A	17,15 (2)	26,16 (3)	-	
B	15,8 (1)	13,22 (0)	+	+
3C	5,5 (4)	11,14 (0)	+	+
4D	18,17 (1)	11,10 (0)	+	+
5C	22,23 (1)	40,27 (84)	+	*mut6*
D	12,9 (2)	9,9 (0)	+	+
6A	30,36 (4)	32,33 (2)	+/-	
D	23,30 (2)	52,45 (0)	+/-	*mut6*
7D	20,14 (2)	8,12 (0)	+	+
8A	20,12 (1)	10,10 (0)	+	+
9A	14,16 (1)	8,5 (0)	+	+
D	45,52 (1)	28,43 (2)	-	*mut6*
10A	13,15 (0)	10,10 (0)	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

δ Only *his1-7* is present in these spores.

TABLE A1: Phenotypes of spores recovered from a cross of XV731-3D/XV731-10A($\frac{mut8}{mut8}$--RO47); and of (h) spores capable of growth at 36°C from cross RO78(*mut8/+*)

Strain RO47-	Prototrophs arising** on limiting histidine[δ]	lysine	Designated mutator
1A	161 (137)	13 (0)	All are *mut8-1*.
B	192 (16)	22 (0)	
C	252 (20)	20 (1)	
D	169 (37)	32 (0)	
2A	167 (39)	15 (0)	
B	164 (62)	22 (0)	
C	322 (41)	30 (0)	
D	260 (23)	28 (0)	
3A	252 (19)	16 (2)	
B	183 (9)	15 (0)	
C	141 (9)	18 (0)	
D	199 (3)	30 (1)	

** Numbers of Prototrophs existing prior to growth on limiting medium are parenthesized.

δ Only *his1-7* is present in these spores.

Strain RO78-	Prototrophs arising** on limiting histidine[δ]	lysine	Designated mutator
1C	33 (0)	10 (0)	+
2B	30 (3)	8 (0)	+
3C	21 (1)	18 (0)	+
D	308 (19)	22 (0)	*mut8*
4C	31 (2)	9 (0)	+
5A	35 (0)	13 (0)	+
D	36 (4)	18 (0)	+
6A	31 (3)	8 (0)	+
7D	27 (0)	15 (0)	+
8A	116 (1)	28 (0)	*mut8*
D	120 (4)	22 (0)	*mut8*
9B	24 (2)	16 (0)	+
C	119 (21)	19 (0)	*mut8*
10A	26 (0)	15 (0)	+
C	1273(1439)*	21 (0)	*mut8*

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

Strains designated as *mut8* were retested (data not shown).

TABLE A1: Phenotypes of spores recovered from cross RO69(*mut9/+*)
(i)

Strain RO69-	Prototrophs arising** on limiting histidine$^{\delta}$		lysine		Growth after γ	Designated mutator(s)	
1A	100	(9)	68	(5)	-	*mut9*	
B	71	(10)	21	(0)	+	+	*mut*
C					+	+	
D	135	(4)	66	(4)	-	*mut9*	
2A	82	(16)	68	(0)	-	*mut9*	*mut*
B	9	(1)	10	(0)	+	+	+
C	10	(1)	12	(0)	+	+	+
D	123	(28)	33	(22)	-	*mut9*	*mut*
3A	71	(15)	15	(0)	+	+	*mut*
B	19	(2)	21	(0)	+	+	+
C	112	(6)	76	(2)	-	*mut9*	
D	89	(4)	67	(2)	-	*mut9*	
4A	102	(16)	62	(58)	-	*mut9*	
B	14	(1)	8	(0)	+	+	+
C	95	(12)	69	(1)	-	*mut9*	
D	44	(4)	33	(0)	+	+	*mut?*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

$^{\delta}$ Only *his1-7* is present in these spores

TABLE A1: Phenotypes of spores recovered from cross RO92($\frac{rad52\text{-}1}{+}$) (j)

Strain RO92-	Segregating alleles at *hom3* *his1*	Prototrophs arising** on limiting lysine		Growth after γ	Designated mutator
1A	All are	19	(0)	+	+
B	*hom3-10*,	58	(0)	-	*rad52*
C	*his1-7*.	66	(0)	-	*rad52*
D		15	(0)	+	+
2A		47	(0)	-	*rad52*
B		40	(3)	-	*rad52*
C		7	(0)	+	+
D		10	(0)	+	+
3A		14	(0)	+	+
B		85	(0)	-	*rad52*
C		17	(0)	+	+
D		43	(1)	-	*rad52*
4A		12	(0)	+	+
B		37	(3)	-	*rad52*
C		15	(0)	+	+
D		65	(1)	-	*rad52*
5A		45	(2)	-	*rad52*
B		13	(0)	+	+
C		3	(0)	+	+
D		87	(25)	-	*rad52*
6A		16	(0)	+	+
B		47	(61)	-	*rad52*
C		72	(1)	-	*rad52*
D		12	(0)	+	+
7A		9	(0)	+	+
B		46	(61)	-	*rad52*
C		16	(0)	+	+
D		27	(3)	-	*rad52*
8A		15	(2)	+	+
B		43	(3)	-	*rad52*
C		58	(0)	-	*rad52*
D		11	(0)	+	+
9A		10	(0)	+	+
B		14	(0)	+	+
C		56	(0)	-	*rad52*
D		43	(0)	-	*rad52*
10A		59	(0)	-	*rad52*
B		9	(0)	+	+
C		55	(0)	-	*rad52*
D		10	(0)	+	+

**Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

TABLE A2: (a) Spontaneous appearance of histidine prototrophs in diploid control strains for *mut8* complimentation tests with double mutator mutants containing *mut8*

Parents	Pertinent genotypes *mut*	at *his1*	Prototrophs arising** on 1γ histidine	3γ histidine	
RO88-1A	*mut8*	*1-1*	6,4	19,13	(0)
RO88-7A	*mut8*	*1-1*	5,4	20,7	(0)
			5,7	33,28	(0)
RO88-1A	*mut8*	*1-1*	0,1	1,1	(0)
RO105-2B	+	*1-1*	0,1	0,0	(0)
			0,0	2,0	(0)
RO508-3B	+	*1-1*	0,0	1,1	(0)
RO88-7A	*mut8*	*1-1*	0,1	2,2	(0)
			0,0	2,1	(0)
LZ13-1A	+	*1-1*	0,0	1,2	(0)
RO105-2B	+	*1-1*	0,0	0,1	(0)
			1,0	0,2	(0)
RO508-3B	+	*1-1*	0,0	1,0	(0)
LZ13-2C	+	*1-1*	1,0	2,1	(0)
			0,0	0,0	(0)
LZ13-1A	+	*1-1*	0,1	4,1	(0)
LZ13-2C	+	*1-1*	0,0	2,2	(0)
			0,0	3,0	(0)
LZ13-1A	+	*1-1*	0,0	1,2	(0)
KF179-15A	+	*1-1*	0,0	1,0	(0)
			0,0	0,1	(0)
LZ13-1A	+	*1-1*	0,0	0,0	(1)*
RO428-6B	+	*1-1*	0,0	1,1	(0)
			0,0	0,2	(0)
RO78-3D	*mut8*	*1-7*	149,210		(346)
RO78-8D	*mut8*	*1-7*	121,150		(202)
			217,200		(190)
RO78-3D	*mut8*	*1-7*	22,31		(24)
RO105-1A	+	*1-7*	26,25		(3)
			21,8		(3)
RO78-8D	*mut8*	*1-7*	5,9		(23)
RO400-10C	+	*1-7*	5,11		(15)
			9,15		(23)

** Numbers of prototrophs existing prio to growth on limiting medium are parenthesized. * 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

TABLE A2: A. Verification of the presence of *mut1* in presumptive (b) *mut7mut1* strains, by testing for diploid complementation with *mut1* testers††

Haploid Parents: Presumptive *mut7mut1* strain	*mut1* tester strain	Prototrophs arising** in diploids on limiting lysine	
RO81-2A	RO71-7C	330,351	(124)
		376,346	(1)
-3C	-1D	1850,1850	(1654)*
		197,201	(207)
-4C	-1D	386,383	(73)
		306,329	(21)
-5C	-1D	859,804	(692)*
		757,783	(693)*
-6A	-1D	398,325	(4)
-7B	-7C	104,107	(56)
-10B	-7C	214,196	(253)
		217,271	(41)
-11A	-1D	154,160	(21)
		1439,1361	(1362)*

B. Verification of the presence of *mut8* in presumptive *mut8mut1* strains, by testing for diploid complementation with *mut8* testers†

Haploid Parents: Presumptive *mut8mit1* strain	*mut8* tester strain	Diploid *his1* Genotype	Prototrophs arising in diploids** on limiting histidine: 1γ histidine		3γ histidine	
RO101-1B	RO88-1A	1-1 / 1-1			12,5	(0)
					16,12	(0)
-2D	-1A	1-1 / 1-1			13,9	(0)
					10,11	(0)
-7C	RO78-8D	1-7 / 1-7	497	(434)*		
			432	(389)*		
-8B	-3D	1-7 / 1-7	159	(436)		
			153	(342)		
-10B	-8D	1-7 / 1-7	394	(608)*		

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

†† See Table 78 for controls.

† See Table A2(a) for controls.

TABLE A2: A. Verification of the presence of *mut2* in presumptive (c) *mut7mut2* strains, by testing for diploid complementation with *mut2* testers††

Haploid Parents: Presumptive *mut7mut2* strain	*mut2* tester strain	Prototrophs arising** in diploids on limiting lysine		Growth on MMS
RO82-1B	RO72-6A	85,97	(1)	-
		87,106	(181)	-
-2C	-1C	120,115	(6)	-
		811,769	(782)*	-
-3C	-1C	132,124	(2)	-
		124,122	(2)	-
-5A	-1C	95,91	(2)	-
		97,115	(3)	-
-6B	-1C	68,119	(17)	-
		620,675	(647)*	-
-8B	-6A	86,94	(11)	-
		116,102	(4)	-
-9C	-6A	40,59	(14)	-
		100,101	(6)	-
-10B	-6A	113,106	(0)	-

B. Verification of the presence of *mut8* in Presumptive *mut8 mut2* strains, by testing for diploid complementation with *mut8* testers†

Haploid Parents: Presumptive *mut8mut2* strain	*mut8* tester strain	Diploid *his1* genotype	Prototrophs arising** in diploids on limiting histidine: 1γ histidine		3γ histidine	
RO102-1B	RO88-7A	1-1 / 1-1			17,11	(0)
					15,11	(0)
-2B	-7A	1-1 / 1-1			17,7	(0)
					20,18	(0)
-4D	RO78-3D	1-7 / 1-7	102	(257)		
			230	(105)		
-6A	RO88-7A	1-1 / 1-1			8,16	(0)
					12,11	(0)
-7B	RO78-3D	1-7 / 1-7	161	(154)		
			213	(165)		
-10B	RO88-7A	1-1 / 1-1			9,12	(0)
					12,19	(0)

**,* See the preceeding table.
†† See Table 78 for controls. † See Table A2(a) for controls.

TABLE A2: (d) A. Verification of the presence of *mut3* in presumptive *mut7mut3* strains, by testing for diploid complementation with *mut3* testers ††

Haploid Parents

Presumptive *mut7mut3* strain	*mut3* tester strain	Prototrophs arising** in diploids on limiting lysine		Growth on MMS
RO83-1B	RO73-2A	30,21	(0)	-
		34,30	(1)	-
-4C	-2A	38,42	(82)	-
		45,32	(0)	-
-6A	-2A	40,36	(0)	-
		27,32	(0)	-
-9B	-2A	92,79	(79)*	-
		20,16	(6)	-
-10A	-2A	35,31	(2)	-
		40,33	(0)	-

B. Verification of the presence of *mut8* in presumptive *mut8mut3* strains, by testing for diploid complementation with *mut8* testers†

Haploid Parents

Presumptive *mut8mut3* strain	*mut3* tester strain	Diploid *his1* genotype	Prototrophs arising** in diploids on limiting histidine			
			1γ histidine		3γ histidine	
RO103-1A	RO88-1A	1-1 / 1-1			13,6	(0)
					19,15	(0)
-2B	-7A	1-1 / 1-1			13,15	(0)
					8,20	(1)
-5B	-1A	1-1 / 1-1			17,11	(0)
					18,11	(0)
-6C	-1A	1-1 / 1-1			13,7	(0)
					8,8	(0)
-8C	-7A	1-1 / 1-1			21,17	(0)
					23,14	(0)
-10A	RO78-3D	1-7 / 1-7	228	(61)		
			196	(59)		

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (in these cases unparenthesized numbers include pre-existing prototrophs).

†† See Table 7B for controls. † See Table A2(a) for controls.

TABLE A2: (e) A. Verification of the presence of *mut4* in presumptive *mut7mut4* strains, by testing for diploid complementation with *mut4* testers††

Haploid Parents: Presumptive *mut7mut4* Strain	*mut4* tester strain	Prototrophs arising** in diploids on limiting lysine		Growth on MMS
RO84-1A	RO74-1B	47,33	(6)	-
		47,52	(0)	-
-2A	-1B	40,27	(1)	-
		25,28	(0)	-
-3D	-1B	37,32	(21)	-
		43,38	(4)	-
-5C	-1B	14,25	(4)	-
		40,39	(0)	-
-7A	-1B	13,5	(0)	+
		5,21	(0)	+
-8A	RO74-3A	53,74	(0)	-
		35,53	(0)	-
-9C	-3A	22,43	(0)	-
		27,43	(2)	-
10B	-1B	20,34	(0)	-
		32,39	(0)	-

B. Verification of the presence of *mut8* in presumptive *mut8mut4* strains, by testing for diploid complementation with *mut8* testers†

Haploid Parents: Presumptive *mut8mut4* strain	*mut8* tester strain	Diploid *his1* genotype	Prototrophs arising** in diploids on limiting histidine: 1γ histidine	3γ histidine
RO104-1D	RO88-1A	1-1 / 1-1		12,9 (0)
				7,19 (0)
-3B	-7A	1-1 / 1-1		0,0 (0)
				0,1 (0)
-4A	-1A	1-1 / 1-1		6,11 (0)
				16,26 (0)
-6B	-1A	1-1 / 1-1		18,21 (1)
				20,10 (0)
-8B	-7A	1-1 / 1-1		8,16 (4)
				16,17 (1)

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized. †† See Table 78 for controls.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.) † See Table A2(a) for controls.

TABLE A2: (f) Verification of the presence of *mut8* in presumptive *mut8mut5* strains, by testing for diploid complementation with *mut8* testers†

Haploid Parents

Presumptive *mut8mut5* strain	*mut8* tester strain	Diploid *his1* genotype	Prototrophs arising** in diploids on limiting histidine 1γ histidine	3γ histidine
RO105-1C	RO88-7A	1-1 / 1-1		21,21 (0) 12,16 (0)
-2A	RO78-8D	1-7 / 1-7	573 (463)* 569 (528)*	
-5C	RO88-1A	1-1 / 1-1		10,12 (0) 14,14 (0)
-6A	-1A	1-1 / 1-1		12,13 (0) 13,12 (0)
-8D	-1A	1-1 / 1-1		12,17 (0) 10,16 (0)
-10D	RO78-3D	1-7 / 1-7	97 (141) 188 (83)	

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

† See Table A2(a) for controls.

TABLE A2: Verification of the presence of *mut8* in presumptive *mut8mut8* strains, by testing for diploid complementation with *mut8* testers††

(g)

Haploid Parents

Presumptive *mut8mut8* strain	*mut8* tester strain	Diploid *his1* genotype	Prototrophs arising** in diploids on limiting histidine	
			1γ histidine	3γ histidine
RO106-4D	RO88-7A	1-1 / 1-1		15,13 (0) 10,7 (0)
-5C	RO78-8D	1-7 / 1-7	262 (441) 691 (610)*	
-8A	-8D	1-7 / 1-7	496 (321) 365 (312)	
-10A	-8D	1-7 / 1-7	1098 (79) 812 (943)	
RO512-1C	-8D	1-7 / 1-7	401 (193) 455 (228)	
-2C	RO88-1A	1-1 / 1-1		12,8 (0) 9,6 (0)
-3C	RO78-8D	1-7 / 1-7	141 (205) 331 (220)	
-5B	RO88-1A	1-1 / 1-1		8,5 (0) 8,14 (1)
-6B	-7A	1-1 / 1-1		17,19 (0) 11,6 (0)
-10A	-7A	1-1 / 1-1		10,10 (0) 11,10 (0)

**Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

†† See Table A2(a) for controls.

TABLE A3: Numbers of viable (transferable) spore clones per tetrad in sporulated diploids used in this study

Strain	No. of tetrads dissected	Number of viable spores/tetrad 4	3	2	1	0
RO5* ($\frac{XV379-10A}{XV379-20C}$)	130	10	22	40	33	25
RO31**	11	5	5	1	0	0
RO61	7	5	1	1	0	0
RO62	7	7	0	0	0	0
RO63	7	6	1	0	0	0
RO64	6	6	0	0	0	0
RO65	8	6	1	1	0	0
RO66	8	6	2	0	0	0
RO69	7	4	3	0	0	0
RO71	15	11	4	0	0	0
RO72	16	12	3	1	0	0
RO73	16	10	5	1	0	0
RO74	17	12	5	0	0	0
RO75	16	13	1	1	1	0
RO76	16	15	1	0	0	0
RO78	17	17	0	0	0	0
RO79	16	14	2	0	0	0
RO81	17	15	2	0	0	0
RO82	16	10	6	0	0	0
RO83	17	15	2	0	0	0
RO84	17	11	6	0	0	0
RO85	50	9	27	11	3	0
RO86	32	5	4	18	1	4
RO88	17	9	7	1	0	0
RO89	16	11	3	2	0	0
RO91	16	5	5	6	0	0
RO92	16	15	1	0	0	0
RO93	16	0	8	7	1	0
RO94	17	1	13	3	0	0
RO94 (15°C)	25	7	14	3	1	0
RO101	15	7	6	2	0	0
RO102	16	7	5	2	0	1
RO103	16	11	4	1	0	0
RO104	16	8	5	1	2	0
RO105	28	13	9	3	3	0
RO106	16	8	4	2	2	0
RO107	16	10	5	0	1	0
RO108	15	10	3	1	0	1
RO109	16	10	4	1	0	1

TABLE A3: (continued)

Strain	No. of tetrads dissected	Number of viable spores per tetrad 4	3	2	1	0
RO101	13	10	1	2	0	0
RO112	15	7	6	2	0	1
RO113	26	11	10	4	1	0
RO117	10	0	0	8	2	0
RO118	9	5	2	1	1	0
RO250	24	22	0	1	1	0
RO251	16	15	0	1	0	0
RO252	15	13	1	1	0	0
RO254	6	4	2	0	0	0
RO255	6	5	0	0	1	0
RO400	17	16	1	0	0	0
RO401	25	23	1	1	0	0
RO402	25	21	4	0	0	0
RO403	9	7	2	0	0	0
RO404	8	7	0	1	0	0
RO405	8	3	4	1	0	0
RO406	8	5	3	0	0	0
RO407	17	14	2	1	0	0
RO408	28	26	2	0	0	0
RO409	7	6	1	0	0	0
RO410	5	4	1	0	0	0
RO411	53	27	20	6	0	0
RO412	7	6	1	0	0	0
RO415	31	26	5	0	0	0
RO417	16	14	2	0	0	0
RO419	29	22	4	3	0	0
RO421	8	3	3	2	0	0
RO422	7	1	0	5	0	1
RO423	9	6	2	1	0	0
RO428	33	27	5	1	0	0
RO506	13	10	3	0	0	0
RO507	13	7	6	0	0	0
RO508	17	14	2	1	0	0
RO510	16	3	11	1	1	0
RO511	17	0	12	5	0	0
RO512	13	12	1	0	0	0
RO513	13	8	3	1	0	1
RO514	13	9	2	1	0	1
RO516	84	45	9	24	4	1
RO517	90	75	6	5	2	2

* homozygous *mut?mut8* strain. XV379-10C was donated by S.K.Quah

** the same cross as RO5. RO5 was sporulated and dissected two to three weeks following mating. RO31 was sporulated three days after mating.

TABLE A4: Phenotypes of viable spore clones recovered from cross RO415

($\frac{mut7}{+}$ $\frac{hom3\text{-}10\ his1\text{-}7}{hom3\text{-}10\ his1\text{-}7}$)

Strain RO415-	Segregating alleles at *ade2*	*arg4*	*cry1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	34°C
1A	+	-	-	96 (17)	39 (0)	-	-
B	-	-	+	25 (0)	11 (0)	+	+
C	+	+	+	21 (1)	10 (0)	+	+
D	-	+	-	65 (9)	37 (0)	-	-
2A	+	+	+	21 (1)	10 (0)	+	+
Bp	-	+	-	69 (1)	30 (0)	-	-
C	-	-	+	22 (2)	10 (0)	+	+
D	+	-	-	19 (0)	33 (0)	-	-
3A	-	-	+	25 (0)	7 (0)	+	+
B	-	+	+	96 (4)	32 (0)	-	-
C	+	-	-	25 (6)	27 (1)	-	-
D	+	+	-	18 (0)	10 (0)	+	+
4A	-	-	-	61 (7)	41 (0)	-	-
Bp	-	+	-	41 (1)	33 (0)	-	-
C	+	-	+	7 (0)	7 (0)	+	+
D	+	+	-	21 (0)	13 (0)	+	+
6A†	+	+	-	48 (5)	24 (6)	-	-
B	-	-	-	21 (0)	9 (0)	+	+
C	+	+	+	91 (7)	29 (2)	-	-
D	-	-	+	26 (3)	10 (0)	+	+
7A	-	-	+	116 (1)	40 (0)	-	-
B	-	-	-	19 (2)	10 (0)	+	+
C	+	+	+	16 (0)	11 (0)	+	+
D	+	+	-	19 (2)	34 (1)	-	-
8A	+	-	+	39 (4)	5 (5)	+	+
Bp	-	-	-	34 (0)	19 (0)	-	-
C	-	+	-	67 (0)	32 (0)	-	-
D	+	+	+	32 (0)	8 (0)	+	+
9A	+	+	+	39 (1)	11 (0)	+	+
B	+	+	-	37 (8)	24 (0)	-	-
C	-	+	+	45 (3)	18 (3)	-	-
D	-	-	-	35 (5)	5 (0)	+	+
10A	-	-	+	13 (1)	9 (0)	+	+
B	+	-	+	14 (1)	4 (0)	+	+
C	-	+	-	118 (5)	46 (0)	-	-
D	+	-	+	96 (1)	36 (0)	-	-
11A	+	-	-	158 (5)	56 (0)	-	-
B	-	-	+	2 (3)	6 (0)	+	+
C	-	+	-	19 (0)	5 (0)	+	+
D	+	+	?	93 (5)	31 (0)	-	-

TABLE A4: (continued)

Strain RO415-	Segregating alleles at *ade2*	*arg4*	*cry1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	34°C
12A	-	-	+	52 (1)	14 (0)	+	+
B	+	+	-	94 (1)	44 (0)	-	-
C	-	-	-	53 (2)	12 (0)	-	-
D	+	+	+	23 (0)	8 (0)	+	+
13A	+	-	+	52 (1)	39 (0)	-	-
B	+	+	+	22 (0)	10 (0)	+	+
C	-	+	-	24 (2)	22 (0)	-	-
D	-	-	-	28 (0)	9 (0)	+	+
14A	+	-	-	84 (2)	32 (0)	-	-
B	+	+	-	20 (1)	11 (0)	+	+
C	-	+	+	JP	69 (1)	-	-
D	-	-	+	3 (0)	5 (0)	+	+
15A	+	-	+	51 (3)	47 (38)	-	-
B	-	-	-	10 (0)	8 (0)	+	+
C	+	+	+	27 (0)	13 (0)	+	+
D	-	+	-	65 (1)	32 (0)	-	-
16A	-	+	-	20 (0)	7 (0)	+	+
B	+	-	+	50 (2)	27 (1)	-	-
C	-	-	-	6 (0)	6 (0)	+	+
D	+	+	+	57 (1)	44 (0)	-	-
17A	+	-	-	18 (2)	16 (0)	+	+
B	+	-	-	79 (2)	41 (0)	-	-
C	-	+	+	14 (1)	9 (0)	+	+
D	-	+	+	42 (0)	20 (0)	-	-
18A	+	-	-	13 (3)	10 (0)	+	+
B	-	-	+	39 (2)	22 (1)	-	-
C	+	+	-	1 (1)	2 (2)	+	+
D	-	+	+	54 (1)	30 (0)	-	-
19A	-	-	-	12 (3)	20 (0)	+	+
B	+	-	+	15 (0)	8 (0)	+	+
C	-	+	+	59 (0)	40 (0)	-	-
D	+	+	-	32 (1)	18 (0)	-	-
20A	-	-	+	43 (0)	16 (0)	+	+
B	-	-	-	9 (0)	9 (0)	+	+
C	+	+	-	50 (3)	25 (3)	-	-
D	+	-	+	45 (3)	25 (5)	-	-
21A	+	+	-	14 (1)	7 (0)	+	+
B	+	-	+	16 (0)	22 (1)	-	-
C	-	+	+	7 (0)	14 (0)	+	+
D	-	-	-	42 (4)	20 (0)	-	-

TABLE A4: (continued)

Strain RO415-	Segregating alleles at *ade2*	*arg4*	*cry1*	Prototrophs arising** on limiting histidine	lysine	Survival at 36°C	34°C
22A	-	+	-	87 (4)	39 (0)	-	-
B	+	-	+	11 (1)	8 (0)	+	+
C	+	-	+	11 (17)	24 (0)	-	-
D	-	+	-	7 (0)	6 (0)	+	+
23A	-	+	+	41 (5)	44 (0)	-	-
B	+	-	-	12 (0)	11 (0)	+	+
C	+	-	+	10 (0)	11 (0)	+	+
Dρ	-	+	-	33 (0)	31 (0)	-	-
24A	+	+	-	17 (3)	43 (0)	-	-
B	-	-	+	52 (6)	47 (20)	-	-
C	-	-	-	15 (1)	14 (0)	+	+
D	+	+	+	27 (1)	10 (0)	+	+

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.
ρ These strains failed to grow when plated to YG medium
† Tetrad #5 was a false tetrad.

Summary of Lassie scores from cross RO415 spore clones

lys1-1 reversion	Mean	Standard Deviation	No. of strains Scored	Standard Error
ts strains	33.0	11.1	46	1.7
Non-*ts* strains	9.4	3.5	46	0.6
his1-7 reversion				
ts strains	58.3	31.2	45	4.7
Non-*ts* strains	19.0	11.0	46	1.7

Table A5: Phenotypes of spore clones from a cross of RO428-6B^{r}11 to RO78-3D. This cross is identical to cross RO88 (Table 58) except that RO428-6B^{r}11 is reverted for the *mut7-1* phenotype.

$$\left(\frac{mut7\text{-}1\text{-}11}{+} \quad \frac{+}{mut8\text{-}1}\right)$$

Strain	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		Prototrophs arising** on limiting lysine (locus)	Suggested mutator
1A	+	1-1	8,6	(0)	21,23	(0)		*mut8*
B	-	1-7	25,32	(0)	10,14	(0)		+
C	-	1-7	532,478	(0)	21,23	(0)		*mut8*
D	+	1-1	0,1	(0)	9,9	(0)		+
2A	+	1-1	8,6	(0)	16,18	(2)	3,5	*mut8*
B	-	1-7	187,207	(210)	22,26	(0)		*mut8*
C	+	1-1	0,1	(0)	478,487	(467)*		+
D	-	1-7	17,11	(0)	12,9	(0)		+
3A	+	1-1	0,1	(0)	12,11	(0)		+
B	-	1-7	183,201	(302)	25,16	(0)		*mut8*
C	-	1-7	35,35	(3)	14,11	(0)	3,1	+
D	+	1-1	8,6	(0)	113,120	(95)*	4,0	*mut8*
4A	+	1-1	3,10	(0)	11,13	(0)		*mut8*
B	-	1-7	18,20	(3)	JP			+
C	+	1-1	7,3	(0)	22,19	(1)	4,3	*mut8*
D	-	1-7	18,20	(0)	11,18	(2)		+
5A	-	1-7	294,329	(179)	87,94	(77)*		*mut8*
B	+	1-1	0,1	(0)	9,23	(4)		+
C	-	1-7	132,195	(242)	28,24	(0)		*mut8*
D	+	1-1	0,0	(0)	12,5	(0)		+
6A	+	1-1	0,0	(0)	10,10	(0)	0,2	+
B	-	1-7	9,24	(2)	23,6	(0)		+
C	-	1-7	127,161	(196)	27,33	(1)	5,8	*mut8*
D	+	1-1	6,7	(0)	19,22	(0)		*mut8*
7A	+	1-1	0,0	(0)	14,22	(0)	2,3	+
B	-	1-7	16,22	(1)	8,9	(1)		+
C	+	1-1	3,7	(0)	19,22	(0)		*mut8*
D	-	1-7	227,232	(154)	14,16	(0)	1,3	*mut8*
8A	+	1-1	5,5	(0)	24,15	(0)		*mut8*
B	-	1-7	13,10	(5)	6,15	(0)	0,2	+
C	-	1-7	25,26	(0)	16,18	(0)		+
D	+	1-1	7,3	(0)	15,15	(0)	4,3	*mut8*
9A	+	1-1	1,2	(0)	16,18	(0)	3,3	+
B	-	1-7	550,583	(0)	19,21	(0)		*mut8*
C	-	1-7	488,505	(0)	17,25	(0)		*mut8*
D	+	1-1	0,1	(0)	13,13	(0)		+

TABLE A5 (continued)

Strain	Segregating alleles at *hom3*	Segregating alleles at *his1*	Prototrophs arising** on limiting histidine		Prototrophs arising** on limiting lysine		lysine (locus)	Suggested mutator†
10A	-	1-7	25,26	(0)	10,7	(0)		+
B	+	1-1	6,11	(1)	18,16	(1)	4,0	*mut8*
C	+	1-1	7,9	(0)	22,17	(0)	3,3	*mut8*
D	-	1-7	43,31	(0)	112,99	(81)*		+
11A	-	1-7	422,522	(0)	24,25	(0)	0,2	*mut8*
B	-	1-7	31,42	(0)	13,12	(0)	0,1	+
C	+	1-1	1,0	(1)*	JP			+
D	+	1-1	9,13	(0)	21,28	(0)		*mut8*

** Numbers of prototrophs existing prior to growth on limiting medium are parenthesized.

* 'Jackpot' (In these cases unparenthesized numbers include pre-existing prototrophs.)

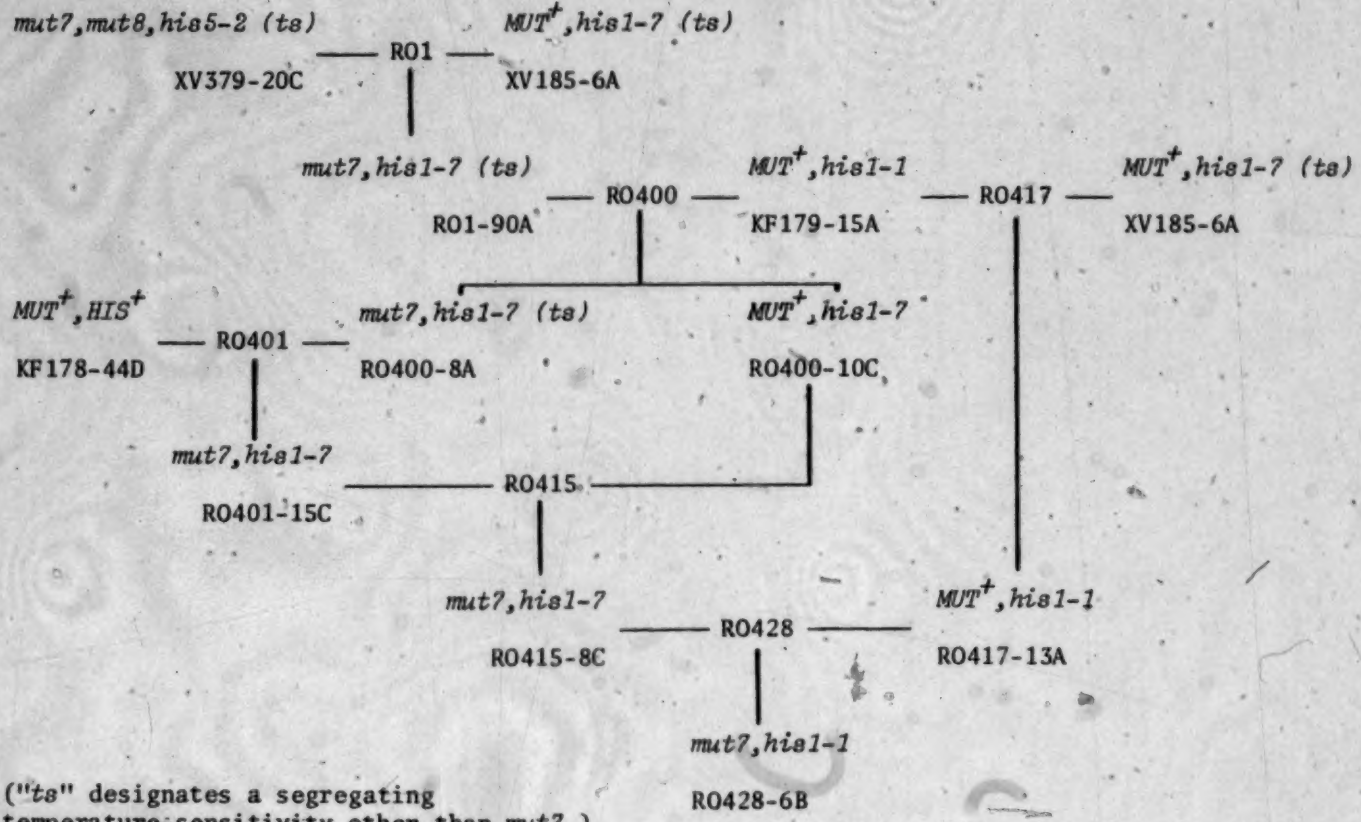
† As determined by relative Lassie scores on limiting histidine.

Controls

RO428-6B		1-1	0,0	(0)	29,42	(0)	10,11	*mut7-1*
RO428-6B^{r}11		1-1	0,1	(0)	13,11	(0)	0,0	*mut7-1-11*
RO78-3D		1-7	614,598	(0)	23,22	(1)		*mut8*
YO300-1C		1-7	7,28	(8)	12,9	(1)		+

Note that no histidine Lassie score exceeds those of the *mut8* parent strain and control, RO78-3D. No lysine Lassie score approaches those of the *mut7-1* control strain, RO428-6B. If *mut7-1* were segregating, the probability that a *mut7mut8* spore clone would not be present in eleven tetrads is less than 0.001, presuming independent assortment of *mut7* and *mut8* as seen from Table 58.

Fig. A1: Crosses made to construct *mut7* stocks free of other temperature-sensitivities, to study the spontaneous reversion of *his1-1*, and to study spontaneous intragenic recombination at *his1*.



("*ts*" designates a segregating temperature-sensitivity other than *mut7*.)

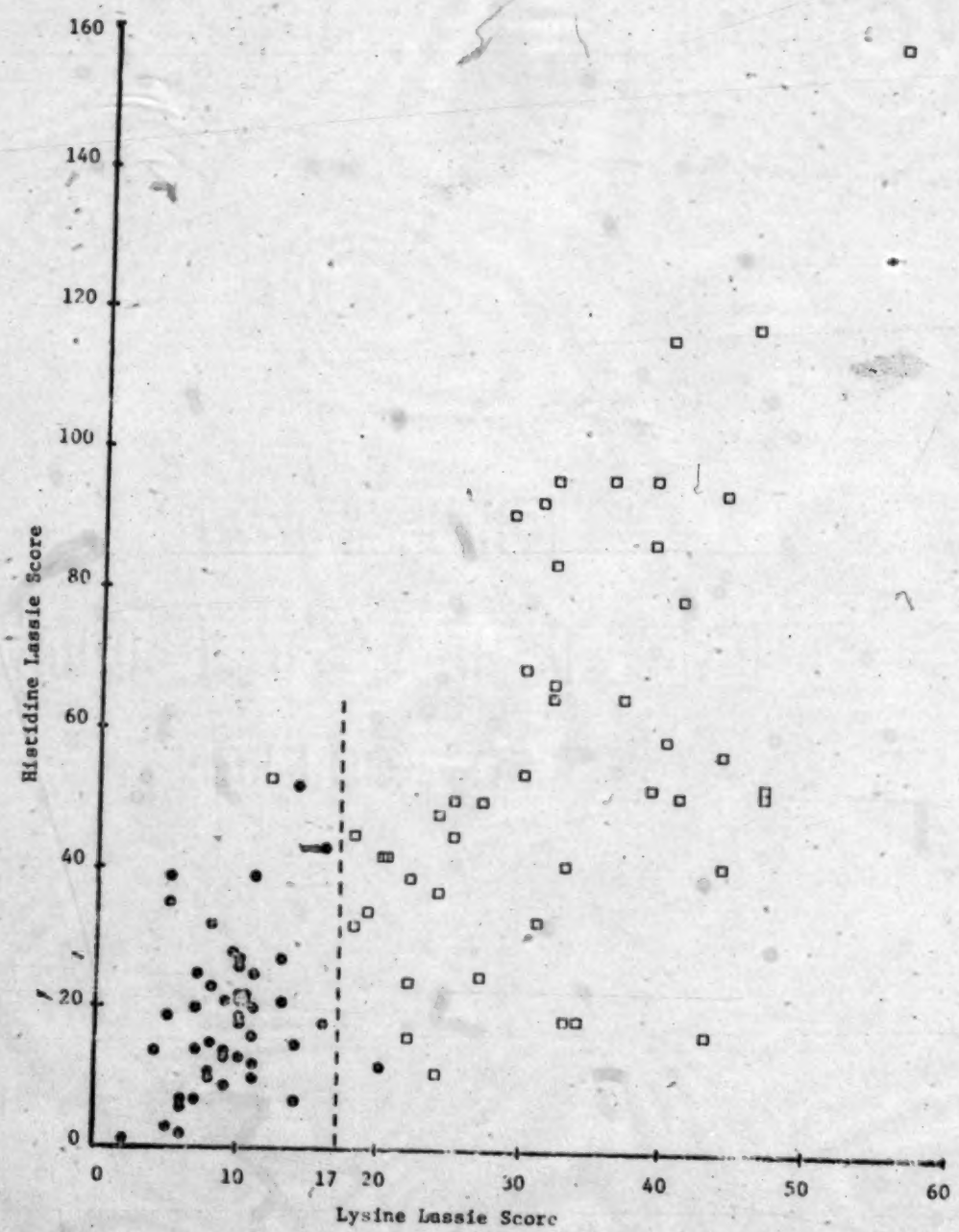


Fig A2: Cosegregation of mutator activity and temperature sensitivity in spore clones from a *mut7/+* diploid. Lassie scores are of *ts* (□) or non-*ts* (•) spore clones from cross R0415 (see Table A4).

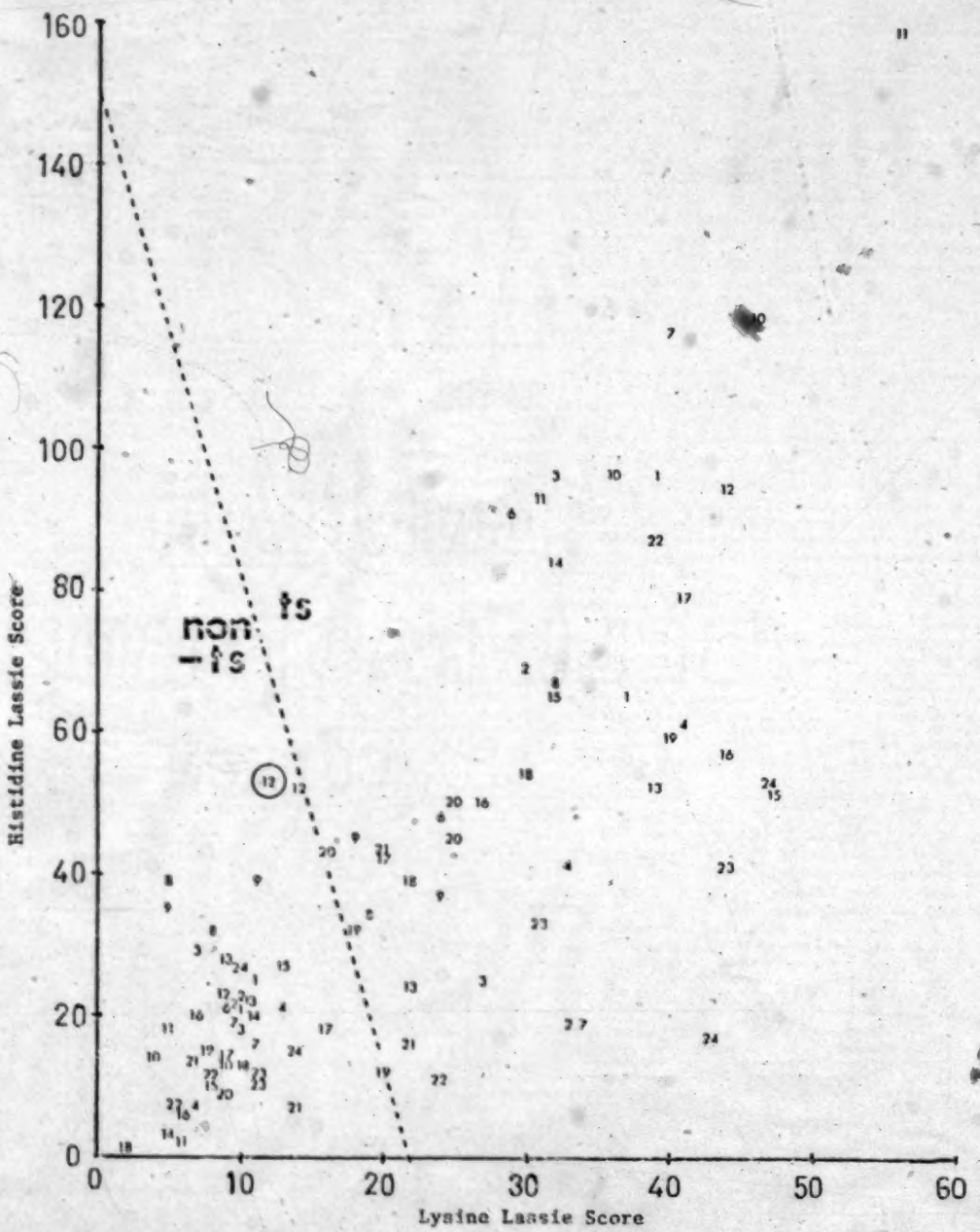


Fig A3: Tetrad numbers from points shown in Figure A2. Strains on or to the left of the dashed line are non-*ts*, excepting strain RO415-12C (circled); strains to the right are *ts*.